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Morphophysiological characterization of *Hymenaea martiana* Hayne seeds and seedlings via manual and digital methods

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Abstract: The application of digital analysis offers great advantages over traditional manual methods because of the shorter time required and greater precision. Given the above, the objective of this study was to perform biometric characterization of *Hymenaea martiana* seeds and seedlings through manual and digital image processing to verify whether the methodology was efficient in distinguishing the physiological qualities of seeds from five mother plants. Seed biometry was performed through manual and digital methods, with length and width being evaluated. In addition, the seed weight and water content were determined. To evaluate physiological quality, the first emergence count, emergence percentage, average emergence index and time, shoot length and root system were measured via manual and digital methods to determine the dry mass of the shoot and root system. The data obtained were subjected to analysis of variance, and the means were compared via Tukey's test at a 5% probability level; subsequently, Spearman's correlation was performed for all combinations of variables. The water content of *H. martiana* seeds was greater in those from mother plants 3 and 5 (11.03 and 10.19%, respectively), whereas the lowest value (7.22%) was in the seeds from mother plant 1; however, there was no significant difference in the water content of the seeds from mother plants 3 and 5.Image analysis, with the aid of the ImageJ® editing program, allows the evaluation of several biometric characteristics of *H. martiana* seeds and seedlings and is efficient in ranking seed lots from different mother plants. The seeds from mother plant 1 are of better quality and are indicated for seedling production.

Keywords: ImageJ®, jatobá, physiological quality.

Introduction

Biometric characterization consists of the morphological evaluation of different parts of a plant, such as fruits and seeds, whose evaluations contribute to the determination of patterns in breeding programs and direct and indirect selection of these characteristics (Zuffo et al., 2019; Smiderle and Souza 2021). In addition, seed biometrics is an important mechanism for detecting genetic variability among populations of the same species and their relationships with environmental factors, thus offering significant support for species differentiation.

Seeds of forest species are usually analyzed via manual methods (calipers) to measure the length, width and thickness of a limited number of seeds, usually fewer than 100 (Correia et al., 2019; Bezerra et al., 2022). Owing to their complexity, these methods are ineffective for large-scale analysis of seeds of one or more plant species. An alternative solution consists of combining digital image processing and seed analysis tools with an image acquisition method, which can optimize the biometric analysis of forest seeds (Felix et al., 2023).

The application of digital analysis offers great advantages over traditional manual methods since manual techniques require time and are sometimes not accurate, being more subject to measurement errors. In contrast, digital image processing offers a variety of information quickly and with significant numbers (Felix et al., 2020). Furthermore, its relevance in the standardization of methodologies to assess the physiological quality of seeds stands out, as it allows differentiation between batches through characteristics, such as various levels of vigor, which enables agility in analyses and the achievement of results with a high level of reliability (Medeiros et al., 2019).

In study digital processing has proven its efficiency for image analysis of seedlings via seeds of forest species (Schneider et al., 2012). In *Leucaena leucocephala* (Lam.) The physiological quality of the seed lots was evaluated via digital image processing with ImageJ® software, and it was possible to distinguish the best lots more efficiently and quickly (Ferreira et al., 2020).

The genus *Hymenaea*, belonging to the Fabaceae family, is found in several Brazilian biomes, such as the Amazon, Caatinga, Cerrado, Atlantic Forest and Pantanal, except in the Pampas. Popularly known as jatobá, this genus comprises trees that occur predominantly in primary dryland forests and stand out for their growth in low-fertility soils (Mojena et al., 2021; Flora do Brasil, 2020).

Species of the genus *Hymenaea* can be used in a variety of ways, in which all their parts can be used, such as their fruits, seeds and leaves, which are used in the pharmaceutical, cosmetic and medicinal industries (Mojena et al., 2021). The wood has economic value and is used

in the manufacture of rafters, beams, slats, door frames, floors, furniture, tool handles, fences, frames, decorative blades, garden furniture, and car bodies, among others (Menezes-Filho et al., 2019).

Hymenaea martiana Hayne, popularly known as "jatobá", is a tree native to the Caatinga of the Fabaceae family. This medicinal plant is distributed throughout Brazil, including the Northeast Region; it has a rounded crown with dense foliage and bark and a straight trunk, is approximately 2 m in diameter, and can be characterized as a large tree that is 15–20 m tall (Shanley and Medina, 2005).

As it is a species of great importance, the propagation of *H. martiana* seedlings in nurseries is relevant to enable the regularization of forest areas that are undergoing reforestation or even the recovery of degraded areas (Sousa et al., 2020), requiring the selection of high-quality seeds.

Given the above, the objective of the present work was to determine the biometric characteristics of *H. martiana* seeds and seedlings through manual and digital image processing, verifying whether the methodology is efficient in distinguishing the physiological qualities of seeds from five mother plants.

Results

Analysis of variance

When the length of *H. martiana* seeds from different mother plants was evaluated via the manual method (Table 1), a greater average (27.30 mm) was observed for those from mother plant 3, whereas the lowest values were found for seeds from mother plants 1 and 5 (23.32 and 22.60 mm, respectively).

Table 1. Biometrics of *Hymenaea martiana* seeds from different mother plants via the manual method and digital image analysis via ImageJ® software

Mother plants	Manual		Digital		Weight (g)
	Length	Width	Length	Width	
	---------- mm ----------				
1	23.32 cd	17.02 b	28.67 c	18.78 c	4.04 c
2	23.65 c	17.00 b	31.07 bc	19.65 bc	3.92 c
3	27.30 a	19.35 a	34.36 a	22.04 a	5.25 a
4	24.80 b	19.42 a	31.36 bc	21.18 ab	5.19 a
5	22.60 d	18.72 a	31.58 ab	21.67 ab	4.49 b
CV (%)	1.82	1.82	4.22	4.87	3.20

Means followed by the same letter in the columns do not differ from each other according to the Tukey test at the 5% probability level. CV= coefficient of variation.

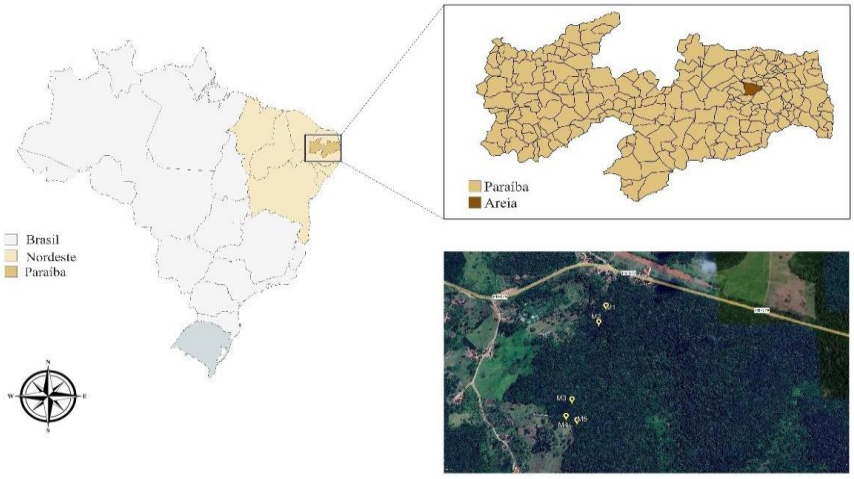

Fig 1. Location of *Hymenaea martiana* mother plants in Mata do Pau Ferro State Park, Areia-PB.

Physical characterization and digital

With respect to the digital method, the seeds from mother plant 3 also stood out in relation to the others, with a higher average, whereas the lowest length values were observed in the seeds from mother plants 4, 2 and 1. For the width variable, the seeds from mother plants 3, 4 and 5 had the highest averages in both evaluation methods, demonstrating that the digital and manual methods were efficient in ranking the lots in terms of biometrics.

The highest average seed weights were observed in those of mother plants 3 and 4, whereas those of mother plants 1 and 2 had lower values, with a reduction in average weight of 20.4% between mother plants with heavier and lighter seeds (Table 1).

Water content

The water content of *H. martiana* seeds (Table 2) was greater in those from mother plants 3 and 5 (11.03 and 10.19%, respectively), whereas the lowest value (7.22%) was in the seeds from mother plant 1; however, there was no significant difference in the water content of the seeds from mother plants 3 and 5.

Table 2. Seed water content (WT), first emergence count (FEC), emergence percentage (EP), emergence speed index (ESI) and mean emergence time (MET) of *Hymenaea martiana* seedlings from different mother plants

Mother plants	WT	FEC	EP	ESI	MET (days)
	---------- % ----------				
1	7.2 b	84 a	93 a	1.04 a	22 b
2	8.8 ab	58 ab	74 b	0.78 b	24 ab
3	11.0 a	54 b	84 ab	0.89 ab	24 ab
4	8.6 ab	41 b	84 ab	0.85 ab	25 a
5	10.2 a	49 b	85 ab	0.86 ab	25 ab
CV (%)	14.24	23.92	9.72	10.64	3.49

Means followed by the same letter in the columns do not differ from each other according to the Tukey test at the 5% probability level. CV= coefficient of variation.

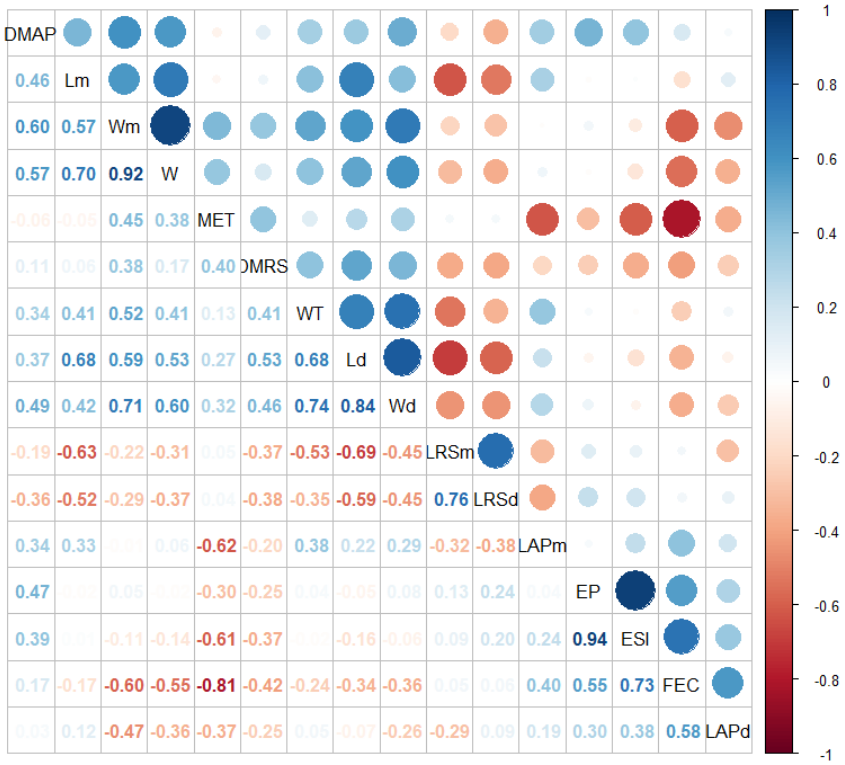

Fig 2. Spearman correlations between the means of the biometric variables of the seeds and the physiological quality of *Hymenaea martiana*. Legend: length via manual and digital methods - Lm and Ld; width via manual and digital methods - Wm and Wd; seed weight - W; seed water content - WT; first emergence count - FEC; emergence percentage - EP; emergence speed index - ESI; mean emergence time - MET; shoot length via manual and digital methods - LAPm and LAPd; root system length via manual and digital methods - LRSm and LRSd; dry mass of the aerial part - DMAP and the root system - DMRS.

Physiological quality

With respect to the physiological quality of *H. martiana* seeds from different mother plants, a greater percentage of emergence was observed in the first seedling count (Table 2) from seeds from mother plant 1, whereas the lowest results were observed in seeds from mother plants 3, 4 and 5.

The highest percentage of seedling emergence (Table 2) occurred when the plants originated from the seeds of mother plant 1, which did not differ statistically from the percentages from the seeds of mother plants 3, 4 and 5, whereas the lowest percentage was obtained from the seeds of mother plant 2.

As shown in Table 2, the highest emergence speed index was greater when the seedlings originated from the seeds of mother plant 1, whereas the seeds of mother plant 2 presented lower performance. On average, the seedlings that originated from the seeds of mother plants 4 and 5 needed a longer time to emerge, whereas the seedlings that originated from the seeds of mother plant 1 needed a shorter time (22.5 days), although it did not differ from that of mother plants 2 and 3, demonstrating that the seeds of this mother plant are highly vigorous.

The length of the aerial parts of the seedlings that originated from the seeds of the different mother plants, as evaluated via both methods, did not vary. In the manual method, the depth ranged from 19.55 cm to 27.47 cm, whereas in the digital method, the width ranged from 14.62 cm to 16.19 cm. In terms of the length of the root system, in the manual method, there was no difference in the means of the seedlings that originated from the seeds of mother plants 1, 2, 4 and 5, whose lengths varied from 13.91 to 14.93 cm; the lowest performance was observed in the seedlings that originated from the seeds of mother plant 3 (9.53 cm). For the digital method, seedlings

originating from the seeds of mother plant 1 had the highest value (10.56 cm), and those of mother plant 3 had the lowest value (7.26 cm) (Table 3).

Table 3. Lengths of the aerial part (LAP) and root system (LRS) of seedlings originating from the seeds of different mother plants of *Hymenaea martiana*, as determined via the traditional method and digital image analysis via ImageJ® software.

Mother plants	Manual		Digital	
	LAP	LRS	LAP	LRS
	---------- mm ----------			
1	24.27 a	14.93 a	16.19 a	10.56 a
2	22.00 a	12.29 a	16.09 a	8.93 ab
3	27.47 a	9.53 b	15.88 a	7.26 b
4	19.55 a	14.46 a	14.62 a	9.21 ab
5	21.36 a	13.91 a	14.71 a	9.69 ab
CV (%)	23.11	9.39	5.60	16.46

Means followed by the same letter in the columns do not differ from each other according to the Tukey test at the 5% probability level. CV= coefficient of variation.

Table 4. Dry mass of the aerial part (DMAP) and root system (DMRS) of seedlings originating from the seeds of different mother plants of *Hymenaea martiana*.

Mother plants	DMAP	DMRS
	---------- g seedling^{-1} ----------	
1	1.09 ab	0.21 b
2	0.98 b	0.27 a
3	1.16 a	0.21 a
4	1.12 a	0.25 ab
5	1.11 a	0.30 a
CV (%)	4.81	9.41

Means followed by the same letter in the columns do not differ from each other according to the Tukey test at the 5% probability level. CV= coefficient of variation.

The results of the dry mass of the aerial part of the seedlings (Table 4) verified that the highest values occurred in those originating from the seeds of mother plants 3, 4 and 5. Similar behavior was observed for the dry mass of the root system, whose greatest accumulation of dry mass was of the seedlings originating from the seeds of mother plants 2, 3 and 5.

Spearman's correlation between the biometric characteristics and the physiological quality of the seeds

The values obtained for Spearman's correlation (Figure 2) among the 16 characteristics evaluated indicated a significant and positive correlation between the biometric characteristics and the physiological quality of the seeds. Thus, seeds with greater lengths tend to have greater widths, weights and water contents. On the other hand, there was a negative correlation between the length of the seed and the length of the root system. In terms of width, positive correlations were observed with the weight, water content and dry mass of the aerial part, whereas the first emergence count was negatively correlated.

For the weight of the seeds, a negative correlation was observed with the first count (-0.6); the greater the weight was, the lower the first emergence count, whereas for the dry mass of the aerial part, there was a positive correlation (0.6), in which as one variable increased, the other variable also increased (Figure 2).

As shown in Figure 2, there was a significant correlation between the variables related to emergence, in which the higher the first emergence count was, the higher the final emergence percentage, the higher the speed index and the shorter the average time for seedlings to emerge. In addition, the shorter the average emergence time is, the greater the length of the aerial part.

Discussion

The biometric variation of seeds is associated with the intraspecific characteristics of the fruits, as they are exposed to several environmental factors, irregular pollination between inflorescences, and strategies for the use of nutrients and water resources available to the plant. Other factors that can also affect these variations are the genotypic diversity of the populations themselves, favoring these phenotypic differences, since the effect of the edaphoclimatic conditions of the region during the development of the seeds directly reflects variations in their shape as well as in their length, width and thickness, weight and even their physiological and health potential (Correia et al., 2019; Barros et al., 2019).

When the seeds of *Pytyrocarpa moniliformis* Benth. Luckow & R.W. Jobson were morphologically evaluated through image analysis (ImageJ®), Felix et al. (2020) obtained satisfactory results, demonstrating the efficiency of this software in measuring and detecting biometric differences between the seeds of different parent plants. The digital methodology was effective and accurate in obtaining estimates for biometric analysis of *Hymenaea stignocarpa* Mart. ex Hayne seeds, making it possible to replace inference analyses with this methodology to minimize errors (Pecegueiro et al., 2020).

The highest average seed weights were observed in those of mother plants 3 and 4, whereas those of mother plants 1 and 2 had lower values, with a reduction in average weight of 20.4% between mother plants with heavier and lighter seeds. Typically, larger seeds have a greater volume of reserves that accumulate throughout their growth, which influences their physiological quality (Carvalho and Nakagawa, 2012). When 80 mother plants of *H. martiana were evaluated*, Silva et al. (2022) reported that for seed weight, ten groups were formed, in which the highest average was 6.90 g and the lightest seeds presented a variation of 2.67 to 2.90 g.

Seed conservation is strongly linked to water content since it directly influences physiological processes, with a loss of seed quality, which can directly affect vigor or even germination power (Marcos-Filho, 2015). In the present study, variation in the water content of seeds was observed among the mother plants, corroborating the work carried out by Smiderle and Souza (2021) when evaluating the water content of *Hymenaea courbaril* L. seeds. Large seeds (mass between 4.9 and 6.4 g) accounted for an average of 10.1%, and small seeds (mass between 2.9 and 4.7 g) accounted for 10.5%. Bezerra et al. (2022), when 19 mother plants of *Erythrina velutina* Willd. The water content of the seeds varied from 10.15% (mother plant 13) to 3.11% (mother plant 9).

With respect to the physiological quality of *H. martiana* seeds from different mother plants, a greater percentage of emergence was observed in the first count of seedlings from mother plant 1, whereas the lowest results were observed in seeds from mother plants 3, 4 and 5. The first count is an extremely important test for assessing germination speed, since the higher the germination rate is in the first count, the greater the vigor of the seeds (Krzyzanowski et al., 2020).

Biotic and abiotic factors influence seed development, and their genetic variability can influence both within and between the same species (Lucena et al., 2017), as observed in the present study, i.e., a variation in the emergence percentage among the mother plants. When the physiological qualities of different batches of *Moringa oleifera* Lam seeds were evaluated, 86 to 88% germination was observed among batches 1, 2, and 4, whereas only 48% germination was observed in batch 3 (Pereira et al., 2020).

To identify batches with faster emergence in the field or even in a greenhouse, the speed index or the first emergence count can be used, thus reducing adverse conditions that occur during germination and seedling establishment (Nascimento and Pereira, 2007). The mother plant obtained the highest speed index and required less time to emerge.

When evaluating the physiological quality of seeds from 28 mother plants of *Poincianella pyramidalis* [Tul] L.P. Queiroz, Lima et al. (2014) reported that seeds from seven mother plants were superior in terms of the germination speed index, with values between 2.039 and 3.779 days, whereas the values of seeds from the other mother plants were between 0.812 and 2.121 days. The emergence test and emergence speed index are efficient at distinguishing the vigor of seeds from different batches of *Moringa oleifera* (Pereira et al., 2020), as occurred in the present study.

In both the manual and digital methods, the seeds from mother plant 1 produced seedlings with a longer root system, whereas those from mother plant 3 presented the lowest values. Although the rankings were similar, the values obtained between the methods were different. In general, in some readings, depending on the shape of the seed, the data estimated with the caliper may change due to a structure that affects the exact reading. This occurs when the seed or fruit has parts that should not be estimated but that go unnoticed and ultimately influence the estimate. However, with the help of digital magnification, it is possible to avoid these common errors caused by the wear and tear of traditional analyses (Pecegueiro et al., 2020), which would explain the variation in values between the manual and digital methods.

The dry mass of the aerial part and the root system varied among the mother plants. According to Bewley and Black (2012), reserve substances accumulate to provide energy and basic substances for the development of the germination process. Therefore, a lower accumulation of dry mass would result in lower seed vigor and lower seedling development than would a greater accumulation of reserves. When the dry mass of the aerial part of *Zizyphus joazeiro* Mart. Lucena et al. (2017) reported that of the five mother plants evaluated, three stood out because they presented the greatest averages.

The values obtained for Spearman's correlation between the 16 characteristics evaluated indicated a significant and positive correlation between the biometric characteristics and the physiological quality of the seeds. The use of correlation is essential, as it allows the evaluation of the impact of selection on one characteristic over another, in addition to facilitating the indirect selection of difficult-to-measure characteristics (Zuffo et al., 2016).

When analyzing the correlation between the biometric characteristics of *H. martiana* seeds, Silva et al. (2022) reported that seeds of greater length tend to be wider. For seed weight, a positive correlation was identified with width and thickness, indicating that wider and thicker seeds tend to be heavier.

In view of the above, the possibility of including computerized analysis of seed images in the characterization of seeds and initial growth of *H. martiana* is highlighted. In addition to the consistency of the results with those obtained in traditionally used tests, the use of this system has advantages, such as speed in obtaining results, greater reliability and precision, and less subjectivity of the analysis. Errors due to differences in evaluation criteria used by analysts from different laboratories are avoided.

Furthermore, tests that use high-speed image capture and data processing are the most suitable for providing a high level of precision in seed quality analyses (Souza et al., 2017). Their use also presents additional advantages, such as providing image archiving for later analysis. Currently, Brazilian research has contributed technical and scientific information on the feasibility of using computerized images of seeds and seedlings; however, the implementation of such methods in routine laboratories involved in quality control programs is still needed.

Material and methods

Location of the experiment and collection and processing of seeds

The study was conducted at the Seed Analysis Laboratory of the Department of Plant Science and Environmental Sciences, Center of Agricultural Sciences, Federal University of Paraíba. The fruits of *H. martiana* were collected from five mother plants in Mata do Pau Ferro, located in the municipality of Areia-PB (Figure 1), between December 2021 and January 2022. The mother plants were selected based on good phytosanitary status, in addition to containing sufficient fruits for the analyses. The climate of the region, according to the Köppen-Geiger classification, is type As' (semihumid tropical, with dry summers), with annual precipitation and temperatures with averages of 1,500 mm and 23 °C, respectively (Alvares et al., 2013).

After collection, the fruits of each mother plant were placed in plastic bags and taken to the laboratory, where they were processed using wooden sticks to open the fruits. The excess pulp was then manually removed from the seeds with a serrated knife and then immersed in water for six hours to facilitate the extraction of the pulp residue still present in the seeds. The seeds were then placed in semipermeable polyethylene bags and stored in a natural environment in the laboratory until the experiment was conducted.

Water content

The water content of the seeds was determined from each batch of four replicates with 10 grams of seeds packaged in aluminum capsules, which were previously identified and placed in an oven at 105 ± 3 °C for 24 hours (Brazil, 2009), and the results are expressed as percentages.

Physical characterization

Biometrics via the manual method

For each mother plant, 100 seeds were used to determine their length and width via a digital caliper (precision of 0.01 mm).

Biometrics via a digital method

Seed image processing was based on the capture and digital analysis of images via the ImageJ® editing program, version 1.46 (Ferreira & Rasband, 2012), which uses 100 seeds per mother plant, which were photographed at a distance of 20 cm with a reference template (ruler graduated in millimeters) on a sheet of vinyl acetate foam (40 × 60 cm).

The images were selected, followed by the definition of the reference scale in millimeters, and the length and width of the seeds were analyzed. Finally, the results were exported in Excel® format.

Seed weight

To determine the weight, 100 seeds from each mother plant were used. The weight was obtained via a precision analytical balance (0.001 g), where one seed was weighed at a time.

Physiological quality of seeds

Emergency test

Owing to tegumentary dormancy, the seeds were scarified on the side opposite the hilum with iron sandpaper nº. 120, and then the seeds were asepsised in a 4% bleach solution for five minutes. Sowing was carried out in plastic pots measuring 30 cm in diameter and 22 cm in height, which were filled with sand sterilized in an autoclave at 120 °C for 120 minutes, leaving 5 cm to the edge, using four replicates of 25 seeds, one pot per replicate, and the seedlings were watered daily with the aid of a watering can.

The test was conducted in a greenhouse, with sowing carried out at a depth of 3.0 cm. The average temperature during the experimental period was 31.7 °C, and the relative humidity was 60%. Seedling emergence was considered when the cotyledons were completely above the substrate. The results were recorded 33 days after sowing and expressed as percentages (Silva, 2020).

First emergency count

The test was carried out together with the emergency test, with a count of the seedlings that emerged 25 days after their installation, with the results expressed as a percentage (Silva, 2020).

Emergency speed index

Its determination was carried out by means of the daily count of the number of emerged seedlings divided by the number of days elapsed between sowing and emergence, according to Maguire (1962).

Average emergency time

This evaluation was carried out in conjunction with the emergence test through daily evaluations of the emerged seedlings, with the results expressed in days and calculated via the formula of Edmond & Drapalla (1958).

Seedling length determination via manual and digital methods

For manual measurement, the length of the aerial part and the root system of each replicate were measured with the aid of a graduated ruler. The length of the aerial part was measured from the collar to the apical meristem, whereas the length of the root system was measured from the collar to the tip of the primary root.

Data regarding the length of the aerial part and the root system via the digital method were obtained via ImageJ® software; ten seedlings per replicate were arranged on a vinyl acetate foam sheet (EVA; 40 × 60 cm) for image capture. The digital processing of the images followed the same protocol mentioned for seed biometry.

Dry mass of aerial parts and the root system

To determine dry mass, normal seedlings from the previous evaluation were separated into shoots and roots, placed in kraft paper bags, identified repeatedly and dried in an ovens at 65 °C for 72 hours. After this period, the samples were weighed on an analytical balance with a precision of 0.001 g, and the results were expressed in g per seedling.

Experimental design and statistical analysis

The experimental design used was completely randomized, with the data obtained subjected to analysis of variance and the means compared by the Tukey's test at a 5% probability level.

Spearman's correlation (rs) was subsequently performed for all combinations between the variables, in which the significance of the rs values was determined by the t test ($P \leq 0.05$). The adjectives to describe the magnitude of the correlations were performed via the Davis methodology (1971), where r = 0.01 to 0.09 are insignificant correlations, r = 0.10 to 0.29 are low, r = 0.30 to 0.49 are moderate, r = 0.50 to 0.69 are substantial, r = 0.70 to 0.99 are very high and r = 1.0 is the perfect correlation. All analyses were performed via R 4.2.1 software (R Core Team, 2022).

Conclusion

Image analysis, with the aid of the ImageJ® editing program, allows the evaluation of several biometric characteristics of H. martiana seeds and seedlings and is efficient in ranking seed lots from different mother plants. The seeds from mother plant 1 are of better quality and are suitable for seedling production.

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