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Thousand-seed weight determination in forest species by image analysis

Francival Cardoso Felix[1], Felipe Ligeski Mocelim[1], Salvador Barros Torres[2]*, Dagma Kratz[1], Richardson Ribeiro[3], Antônio Carlos Nogueira[1]

ABSTRACT: Thousand-seed weight (TSW) is used to calculate the sowing density, number of seeds present in a sample, and predict seed size. Our aim was to establish an alternative methodology for thousand-seed weight determination in forest species using image analysis techniques. The traditional methodology was compared with the alternative one, testing them on seeds from 16 forest species. The traditional method was performed by manually counting eight repetitions of 100 seeds, weighing of samples, and computation of thousand-seed weight. The alternative methodology was performed by counting seed samples captured by images and processing in ImageJ® software, followed by weighing a single sample, and computation. All steps were timed, and each methodology was repeated ten times per species. The TSW obtained by the alternative methodology was similar to that obtained by the traditional one, and the average execution time of the activities was reduced by 62%. The proposed thousand-seed weight determination by image analysis is an efficient and optimized alternative to the traditional method. Thousand-seed weight determination from image analysis reduces execution time for the seed analyst and forest seedling producer.

Index terms: ImageJ, physical analysis, physical determination, Rules for Seed Testing, seed count.

RESUMO: O peso de mil sementes (PMS) é utilizado para calcular a densidade de semeadura, estimar o número de sementes presente em uma amostra e predizer o tamanho das sementes. Objetivou-se estabelecer uma metodologia alternativa para determinação do peso de mil sementes em espécies florestais usando técnicas de análise de imagens. A metodologia tradicional foi comparada com a alternativa, testando-as em sementes de 16 espécies florestais. O método tradicional foi feito com a contagem manual de oito repetições de 100 sementes, pesagem das amostras e cálculo do peso de mil sementes. A metodologia alternativa foi executada por meio da contagem de amostras de sementes capturadas por imagens e processamento no software ImageJ®, seguido da pesagem de uma amostra única e cálculo. Todas as etapas foram cronometradas e cada metodologia foi repetida dez vezes por espécie. O PMS obtido pela metodologia alternativa foi semelhante à tradicional, e o tempo médio de execução das atividades foi reduzido em 62%. Portanto, a determinação do peso de mil sementes proposto por análise de imagens constitui-se em alternativa eficiente e otimizada em relação ao método tradicional. A determinação do peso de mil sementes a partir da análise de imagens proporciona redução do tempo de execução para o analista de sementes, produtor de sementes e de mudas florestais.

Termos para indexação: ImageJ, análise física, determinação física, Regras para Análise de Sementes, contagem de sementes.

***Corresponding author**
E-mail: sbtorres@ufersa.edu.br

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[1]Universidade Federal do Paraná (UFPR), 80210-170, Curitiba, PR, Brasil.

[2]Universidade Federal Rural do Semi-Árido (UFERSA), 59625-900, Mossoró, RN, Brasil.

[3]Universidade Tecnológica Federal do Paraná (UTFPR), 85503-390, Pato Branco, PR, Brasil.

INTRODUCTION

Determinations of physical properties in the analysis of forest seeds are fundamental for the production and commercialization process, directing storage, sowing and germination (Piña-Rodrigues et al., 2015). Among the main physical analyses, thousand-seed weight (TSW) is used to compute the sowing density, estimate the number of seeds present in a sample, predict seed size, and determine the working sample for purity analysis and germination test when not specified in the Rules for Seed Testing (RAS) (Brasil, 2009) or International Rules for Seed Testing (ISTA) (ISTA, 2019).

The traditional methodology of thousand-seed weight basically consists of the manual or mechanical counting of eight replications of 100 seeds, with subsequent weighing on scale and application of mathematical formula (Brasil, 2009; ISTA, 2019), which requires time to perform these three activities, particularly for visual counting of seeds by the analyst. The evaluation of seed lots by the traditional methodology associated with other laboratory activities becomes exhaustive. Therefore, the application of tools that optimize time and improve the technique is fundamental.

In Brazil, 35,552 species of Angiosperms and 114 species of Gymnosperms are catalogued (Reflora, 2020), and it is virtually impossible to have recommendations for tests and laboratory analyses for each one. Therefore, producers of seeds and forest seedlings face particularities due to the large number of species that represent the biodiversity of Brazilian ecosystems. In addition, they do not have sophisticated seed counting equipment and machinery, such as those for agricultural species.

Moreover, the manual or visual counting of seeds is tiring, repetitive, inefficient and increases the chances of human error, which can be overcome with the use of image processing techniques (Mussadiq et al., 2015; Acosta et al., 2017; Deng et al., 2021). ImageJ® is a free access software with several applications in image analysis in agrarian and biological sciences (Noronha et al., 2019; Freitag et al., 2020; Medeiros et al., 2020; Silva et al., 2020; Oliveira et al., 2021; Ribeiro et al., 2021). Among them, the possibility of counting elements in an image for different purposes (Passoni et al., 2014; Gautier and Ginsberg, 2021; Tsuzuki et al., 2021).

Therefore, the objective of this study was to establish an alternative methodology for determining the thousand-seed weight in forest species using image analysis techniques for seed counting. Therefore, we seek to answer the following questions: (i) does the proposed approach have the same effectiveness as the traditional methodology? (ii) is the execution time of activities reduced or optimized as compared to the traditional methodology?

MATERIAL AND METHODS

The seed lots used in this study came from the seed bank of the Laboratory of Forest Seeds of the *Universidade Federal do Paraná*, Curitiba, Paraná, Brazil. The moisture content of each seed lot was previously measured by the oven drying method at 105 ± 3 °C / 24 h with two replications of 4.5 ± 0.5 g, as recommended by the RAS (Brasil, 2009), whose results were expressed as percentage.

The traditional methodology to determine thousand-seed weight was compared with the alternative methodology based on image analysis techniques (Figure 1), testing them in 16 forest species with seeds with different characteristics, sizes and shapes: *Anadenanthera colubrina* (Vell.) Brenan (Fabaceae), *Calopogonium mucunoides* Desv. (Fabaceae), *Cassia leptophylla* Vogel (Fabaceae), *Ilex paraguariensis* A.St.-Hil. (Aquifoliaceae), *Jatropha curcas* L. (Euphorbiaceae), *Mimosa bimucronata* (DC.) Kuntze (Fabaceae), *Mimosa pigra* L. (Fabaceae), *Mimosa scabrella* Benth. (Fabaceae), *Mucuna pruriens* (L.) DC. (Fabaceae), *Peltophorum dubium* (Spreng.) Taub. (Fabaceae), *Pinus elliottii* Engelm. (Pinaceae), *Pinus taeda* L. (Pinaceae), *Psidium cattleyanum* Sabine (Myrtaceae), *Schizolobium parahyba* (Vell.) Blake (Fabaceae), *Senna multijuga* (Rich.) H.S.Irwin & Barneby (Fabaceae) and *Solanum viarum* Dunal (Solanaceae).

Traditional methodology

The thousand-seed weight determined by the traditional methodology described in the Rules for Seed Testing and International Rules for Seed Testing (Brasil, 2009; ISTA, 2019) followed three steps (Figure 1): (1) manual count of eight replications of 100 seeds of the pure seed portion; (2) weighing of samples on a scale (0.001 g); and (3) calculation of thousand-seed weight. The TSW was calculated in a data spreadsheet (Microsoft Excel®) by filling cells in a pre-established table, obtaining by estimation the weight in grams of one thousand seeds.

Alternative methodology

The alternative methodology based on image analysis was also performed in three steps (Figure 1): (1) counting of the number of seeds present in a random sample (Table 1) by capturing digital images and processing using ImageJ® software (Ferreira and Rasband, 2012); (2) weighing a single sample on a scale (0.001 g); and (3) calculation of thousand-seed weight. The TSW was calculated in a data spreadsheet (Microsoft Excel®) by filling cells in a pre-established table, obtaining by estimation the weight in grams of one thousand seeds. For the imaging technique, the steps of image acquisition, pre-processing and processing are described below:

Image acquisition: the seeds of each species were spatially arranged on 40 x 50 cm ethylene-vinyl acetate (EVA) foam sheet, and the images were captured with Apple iPhone X smartphone camera (12MP lens) at a distance of 50 cm from the seeds (Figure 1A) under artificial LED light. For each species, EVA sheets with colors (white or black) contrasting to the seed color were used, that is, dark seeds were photographed on white EVA and light seeds were photographed on black EVA. The minimum lighting conditions should allow the differentiation of the seeds from the background (EVA foam) and can be obtained in any illuminated environment.

Preprocessing: Images were transferred to a microcomputer and processed in ImageJ® software (https://imagej.nih.gov/ij/). The images used were the originals in JPEG format. If there were elements of the image besides the seeds and background (EVA foam), these were removed/clipped from the image prior to processing.

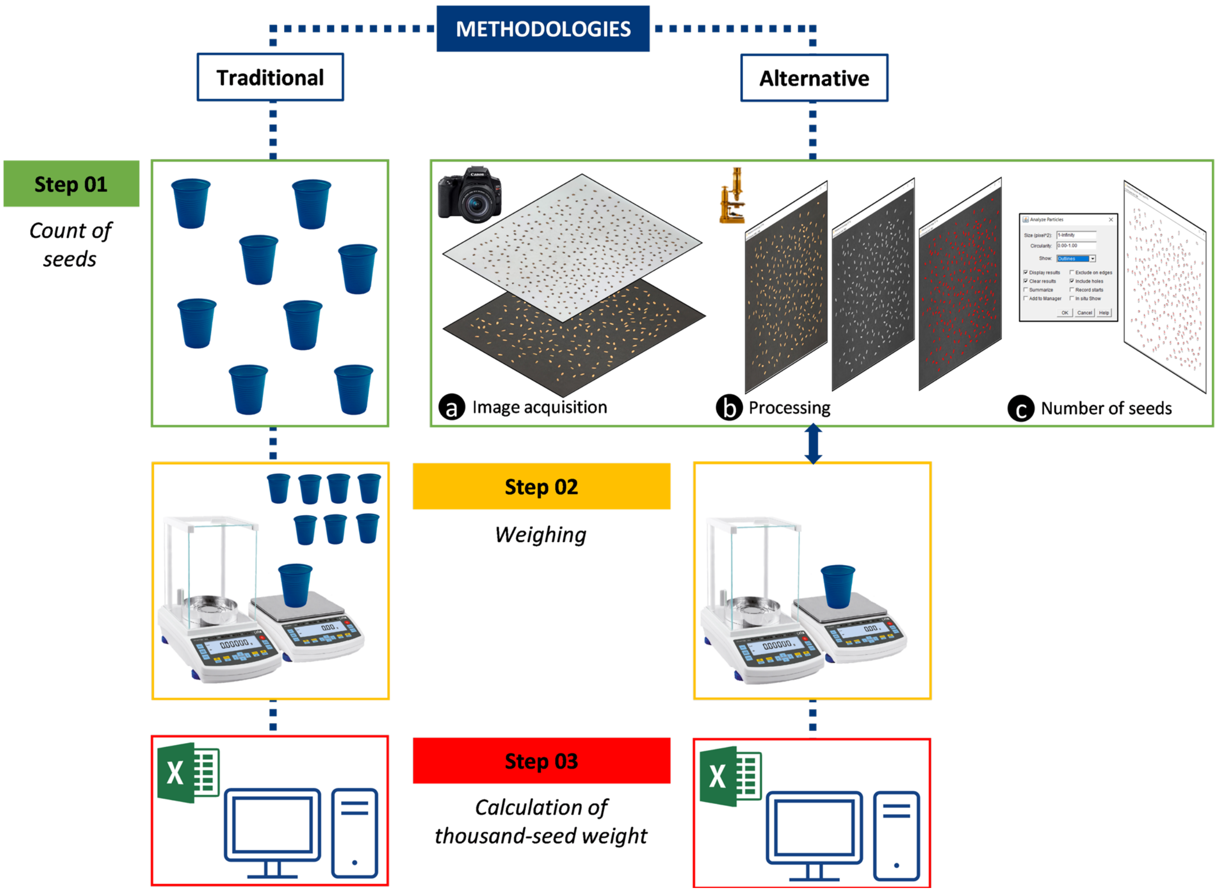

Figure 1. Scheme of execution of the traditional methodology and alternative methodology by image analysis to determine the thousand-seed weight for forest species.

Processing: images were processed in ImageJ® in the following steps: (i) image opening; (ii) conversion to 8-bit format; (iii) application of the *Threshold* mask (Figure 1B); and (iv) particle analysis (Figure 1C). The software computes the number of seeds present in the sample based on the area occupied by each seed in the image. For this, the area measurement option was activated, following *Analyze* and *Set Measurements*.

Times and movements and statistical analysis

The steps of counting the number of seeds (Step 1), weighing (Step 2) and calculating the weight of one thousand seeds (Step 3) were timed in each methodology (Figure 1), thus obtaining the time (s) of execution of the activities. For each species, the traditional methodology and the alternative methodology were tested ten times with new samples in each replication. The *t*-test was applied to compare the methodologies at 5% probability level. Finally, multivariate analysis of principal components was performed to verify the relationship between the methodologies and the evaluated species. The statistical software used was Past® (version 3.8) (Hammer and Harper, 2008).

RESULTS AND DISCUSSION

The forest species evaluated have seeds of different shapes, sizes and weights, as verified by the different values of TSW (Table 1). The thousand-seed weight obtained by the alternative methodology was statistically similar to that obtained by the traditional methodology for all species evaluated. Therefore, counting the seeds by the traditional method and by image analysis makes it possible to achieve similar results, regardless of the size and physical characteristics of the seeds of the species used in this study. In this context, seed counting by images proved to be efficient as an alternative to the traditional methodology.

Table 1. Thousand-seed weight determined by the traditional method and by image analysis (alternative methodology) for 16 forest species.

Species	MC (%)	Thousand-seed weight (g)		F value	CV (%)	*n*
		Traditional	Alternative			
Anadenanthera colubrina	11.9	67.25	67.68	2.110^{ns}	1.00	1,783
Calopogonium mucunoides	10.3	15.09	15.06	0.087^{ns}	1.61	3,487
Cassia leptophylla	12.6	253.32	251.20	0.969^{ns}	1.91	1,686
Ilex paraguariensis	9.2	6.71	6.77	1.446^{ns}	1.63	3,569
Jatropha curcas	7.8	718.01	719.04	0.024^{ns}	2.02	1,046
Mimosa bimucronata	8.2	10.34	10.28	0.635^{ns}	1.36	3,315
Mimosa pigra	6.0	14.34	14.20	3.838^{ns}	1.19	3,029
Mimosa scabrella	12.2	12.31	12.49	1.203^{ns}	2.98	2,736
Mucuna pruriens	10.9	801.08	804.16	0.226^{ns}	1.77	1,508
Peltophorum dubium	9.5	51.47	51.54	0.074^{ns}	1.11	2,169
Pinus elliottii	9.9	33.46	33.37	0.346^{ns}	0.98	3,219
Pinus taeda	10.1	23.59	23.68	0.323^{ns}	1.45	4,457
Psidium cattleyanum	9.0	21.06	21.11	0.051^{ns}	2.26	2,411
Schizolobium parahyba	5.7	946.85	938.81	3.508^{ns}	1.08	1,329
Senna multijuga	12.2	10.94	11.00	0.801^{ns}	1.32	2,856
Solanum viarum	11.7	2.22	2.24	1.408^{ns}	1.55	3,134

MC: Moisture content. CV: coefficient of variation between methodologies. *n*: total size of the sample per species used for image analysis. ^{ns} not significant at 5% probability level by the *t*-test.

The determination of thousand-seed weight by the traditional method takes on average 715 seconds (11:55 min) for each species, being 485 s (8:05 min) for counting the seeds, 191 s (3:11 min) for weighing the samples and 39 s for calculating TSW (Table 2). On the other hand, the execution of the activities by the alternative method required 196 s (3:16 min) for counting the seeds, 39 s for weighing the sample and 35 s for calculating TSW, totaling 270 s (4:30 min). Therefore, the average time of total execution of activities is reduced by 62% (±5%) using the alternative methodology, with reductions of 60% (±8%) for seed counting by images, 80% (±2%) for sample weighing and 9% (±4%) for calculation in a pre-established data spreadsheet.

The number of seeds is pre-established only for the traditional methodology. The proposed method uses one random sample of each lot, eliminating the use of repetitions, since image capture makes it possible to compute different amounts of seeds in a larger sample (Table 1). Therefore, there was a more marked reduction of time in the steps of seed counting and weighing between the traditional and the alternative methodology, in which only one sample needs to be weighed and computed to obtain the TSW. Precisely to ensure that this sampling is effective in comparison to the traditional method, in each species, the methodologies were repeated ten times and no statistical difference was observed, despite the different numbers of seeds used in the alternative methodology.

The use of ImageJ® software makes it possible to compute the number of particles, cells or contrasting elements in the images (Passoni et al., 2014; Gautier and Ginsberg, 2021; Tsuzuki et al., 2021), as well as seeds in a photograph (Mussadiq et al., 2015). Counting rice and wheat grains by means of images was satisfactory and adequate to determine the number of seeds present in a sample, highly correlated with manual counting (Acosta et al., 2017; Deng et al., 2021). Therefore, seed counting by images can be an alternative for the seed analyst and seedling producer.

Table 2. Execution time of the activities thousand-seed weight determination by the traditional method and by image analysis (alternative methodology) for 16 forest species.

Species	Execution time of the activities (s)							
	Traditional TSW				Alternative TSW			
	(1)	(2)	(3)	Total	(1)	(2)	(3)	Total
Anadenanthera colubrina	526	194	43	763	175	47	35	258
Calopogonium mucunoides	485	177	33	695	201	41	33	275
Cassia leptophylla	388	172	46	606	153	39	31	223
Ilex paraguariensis	573	214	42	829	241	44	36	321
Jatropha curcas	434	213	47	695	114	32	33	179
Mimosa bimucronata	482	176	34	692	254	35	32	321
Mimosa pigra	450	161	36	647	212	36	33	282
Mimosa scabrella	693	208	35	935	215	37	37	290
Mucuna pruriens	376	223	56	655	170	37	36	243
Peltophorum dubium	497	200	40	737	180	42	38	259
Pinus elliottii	497	217	37	751	237	42	38	316
Pinus taeda	474	242	37	753	249	48	50	347
Psidium cattleyanum	408	161	31	599	166	34	36	237
Schizolobium parahyba	411	118	41	570	134	25	34	193
Senna multijuga	527	181	29	737	203	40	31	275
Solanum viarum	539	200	31	770	228	38	31	297
Mean	485	191	39	715	196	39	35	270

Steps: (1) counting of the number of seeds; (2) weighing; and (3) calculation of thousand-seed weight. TSW: Thousand-seed weight; s: seconds.

The application of image analysis in seeds has advantages over traditional techniques (Varma et al., 2013), mainly due to the speed and optimization of evaluations (Hemender et al., 2018), as verified in the present study. Fast image processing provides a more efficient, pleasant, and interactive workplace (Kapadia et al., 2017). In addition, image analysis reduces the time required for the execution of activities, optimizing evaluation methodologies, which brings operational advantages and objectivity to the analyses (Peñaloza and Durán, 2015).

Principal component analysis made it possible to differentiate the TSW determination methodologies from component 1 (59.5%), while component 2 (26.6%) is directly related to the seed weight of the evaluated species (Figure 2). It is noticed that species with smaller seeds required more time for counting by the traditional method. The alternative image-based methodology shows less variation in the results when compared to the traditional methodology and, therefore, it proves to be a more precise method regardless of the evaluated species or seed size.

The species *J. curcas* (TSW: 719.04 g), *M. pruriens* (TSW: 804.16 g) and *S. parahyba* (TSW: 938.81 g) obviously have seeds of larger size and weight (Figure 2) and, although smaller amounts of seeds in each sample were used in the alternative method, it was statistically similar to the traditional method (Table 1). On the other hand, small seeds of *S. viarum* (TSW: 2.24 g), *I. paraguariensis* (TSW: 6.77 g), *S. multijuga* (TSW: 11.00 g) and *M. scabrella* (TSW: 12.49 g) required longer counting time by the traditional method compared to the alternative method, again without statistical difference between methodologies (Table 1).

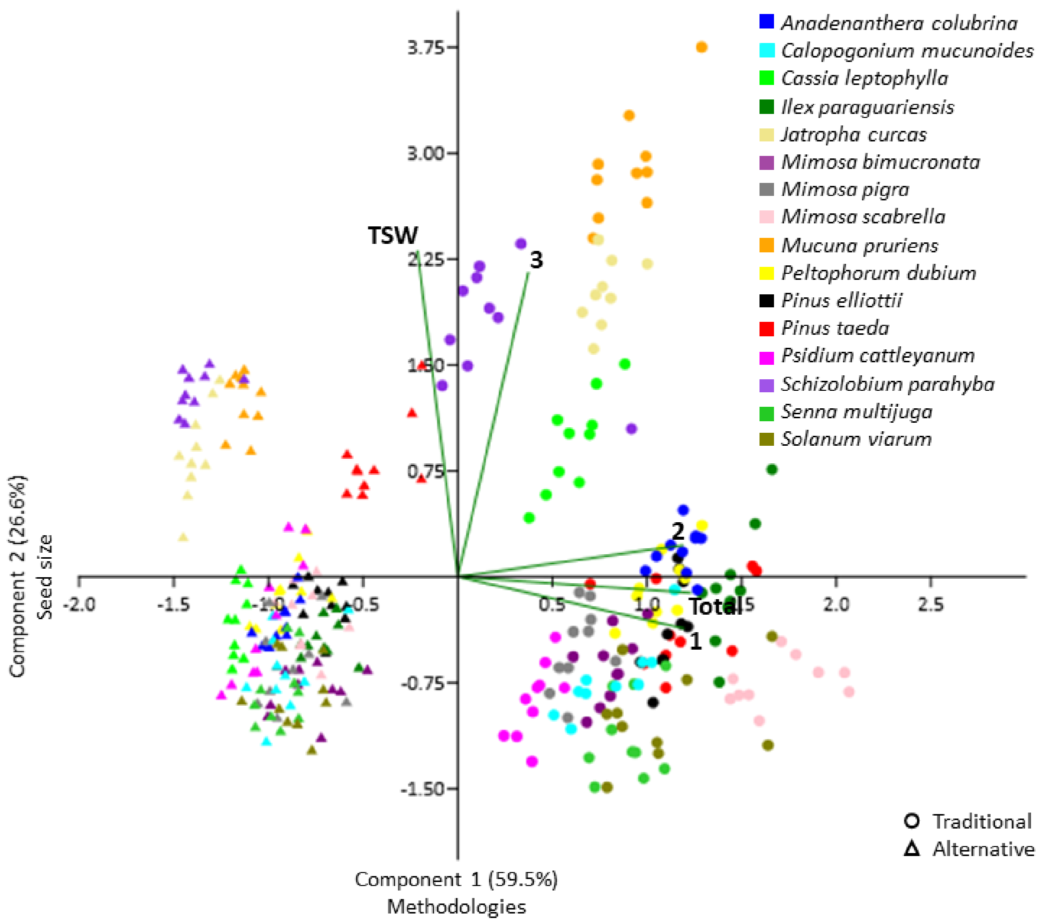

Figure 2. Principal component analysis for the relationship between the thousand-seed weight determination methodologies by the traditional method and by image analysis (alternative methodology) evaluated for 16 forest species. Steps: (1) counting of the number of seeds; (2) weighing; and (3) calculation of thousand-seed weight. TSW: thousand-seed weight.

The use of the alternative methodology suggested in the present study is promising for rapid and efficient determination of thousand-seed weight in native and exotic forest species as an alternative method. This information is useful for estimating the number of seeds present in a seed lot, determining the amount of seeds needed for direct seeding in the field, in the nursery and laboratory analyses, since the official instructions (Brasil, 2009; Brasil, 2013; ISTA, 2019) do not contemplate all forest species due to the diversity of species that occur in Brazil (Reflora, 2020). However, further studies with different species and amounts of seeds can be conducted to increase the reliability of this method to replace the official methodology.

Among the advantages of the proposed method, the following stand out: (i) the steps of seed counting by images and sample weighing can be performed regarded of the order; (ii) possibility of capturing images with various photographic devices; (iii) agility and optimization of steps that save analyst time; (iv) the image processing practice by the analyst may reduce the execution time of this methodology as experience increases; and (v) possibility of obtaining biometric parameters and other variables together.

As limiting factors and disadvantages, the following stand out: (i) the proposed methodology continues to be semi-automated, requiring the analyst to perform the processing of images on a computer; and (ii) thousand-seed weight continues to be difficult to be estimated for very small seeds (TSW: < 1.0 g or 1 million seeds per kilogram) and very large seeds (TSW: > 5,000 g or 200 seeds per kilogram), regardless of the method. Very small seeds do not enable manual counting or distinction from the background for image processing, and impurities interfere in the precision of the method (e.g. eucalyptus). On the other hand, very large seeds would require dozens of photographs, and every 100 seeds would exceed the limit of common laboratory scales (e.g. coconut). However, these groups of species represent exceptions for which the methodologies presented here would need adaptations.

Finally, future studies with the development of mobile applications for capturing, processing, and counting seeds of different sizes and shapes can be an advanced solution to improve the methodology of determining the thousand-seed weight. However, weighing a seed sample is necessary due to the variation of weight according to water content or size of the seed at the time of analysis, which should be performed on scales.

CONCLUSIONS

The proposed determination of thousand-seed weight by counting the seeds through image analysis is an efficient and optimized alternative to the traditional method recommended in the Brazilian and International Rules for Seed Testing. The execution time of the activities is reduced on average by 62% with the alternative methodology, promoting greater agility for the seed analyst and producer of forest seeds and seedlings. However, studies with other species that have less common seeds or diaspores can be conducted to confirm the efficiency of this method.

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