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Digital image processing as a component to assess primary root emergence from soybean seeds

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ABSTRACT: Among the tests available for seed vigor assessment, the primary root emergence (RE) test has gained prominence as a provider of valuable information regarding the seed performance of different species on account of its efficiency, sensitivity and rapidity. Furthermore, the use of computational resources to identify the RE can be very useful for refining the information provided. This research study aimed to evaluate the efficiency of RE, compared to recommended tests assessing the seed vigor of soybean seeds, and verify whether specific parameters, as measured by the ImageJ® software to identify RE, are effective in estimating soybean seed vigor. Nine seed lots with similar germination but differences in vigor were used. Germination and vigor (germination first count, electrical conductivity, accelerated aging, tetrazolium, RE, and field seedling emergence tests were performed); RE was evaluated after germination under four temperature/period combinations. After the RE test, seedling images were captured and analyzed by ImageJ® software and data from the perimeter, area, circularity, aspect ratio, roundness, and solidity were obtained. Information provided by the RE test was similar to those from the accepted vigor tests, indicating the possibility of including this test in soybean seed quality control programs. Parameter circularity, aspect ratio, roundness and solidity provided consistent data for detecting the RE from soybean seeds germinated at 30 °C for 24 h, with a primary root length of at least 1.0 mm. These parameters provide a rapid and objective evaluation of soybean seed vigor.

Keywords: *Glycine max* (L.) Merrill, computer vision, ImageJ, image analysis, physiological potential

Introduction

Speed and uniformity of seedling emergence are crucial factors in assuring high seed performance, and they have a direct influence on field stand establishment. Vigorous seeds exhibit enhanced efficiency in the mobilization of stored reserves. This improves the resumption of metabolism and the action of repair mechanisms just after the start of seed imbibition in contrast with more deteriorated seed lots.

In this context, the slowing of germination as a visible consequence of extended seed aging has been noted (Matthews et al., 2012). The duration of Phase II of the germination process, commonly known as the "lag-phase", is proportional to the degree of deterioration (Matthews and Khajeh-Hosseini, 2007). Based on this understanding, a method was developed to assess the precocity of primary root emergence (RE). Initial investigations into maize (*Zea mays* L.) seeds produced results comparable to those of cold and seedling emergence tests (Toledo et al., 1999). Studies have consistently shown that RE is a reliable indicator of seed vigor across various species, including maize (Matthews and Khajeh-Hosseini, 2007; Matthews et al., 2010; Powell and Matthews, 2012), sweetcorn (*Zea mays* group saccharate) (Alvarenga et al., 2013), radish (*Raphanus sativus* L.) (Mavi et al., 2016), rice (*Oryza sativa* L.) (Luo et al., 2017), eggplant (*Solanum melongena* L.) (Ozden et al., 2018), cauliflower (*Brassica oleracea* var. *botrytis* L.) (Shinohara et al., 2021), melon (*Cucumis melo* L.), lettuce (*Lactuca sativa* L.), and carrot (*Daucus carota* L. subsp. *Sativus*) (Mis et al., 2022).

On the other hand, despite the existence of efficient procedures for assessing seed vigor, including RE, an important improvement in seed testing procedures involving the use of advanced technologies has been observed, particularly with regard to computational resources, consequently refining the information provided. A relatively underexplored option is the utilization of ImageJ® software (National Institutes of Health) for assessing RE through its correlation with morphological parameters, including perimeter, area, circularity, aspect ratio, roundness, and solidity. This research was carried out to verify the efficiency of the ImageJ® software by evaluating morphodimensional attributes that identify the RE and its relationship to soybean (*Glycine max* (L.) Merrill) seed vigor, compared with other recommended and accepted tests.

Materials and Methods

The research was carried out in 2021 in the Laboratório de Análise de Sementes and Laboratório de Análise de Imagens "Professor Silvio Moure Cicero" of the Departamento de Produção Vegetal of the Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo, in Piracicaba (22°42′30″ S, 47°36′00″ W, altitude 546 m), São Paulo state, Brazil.

Nine seed lots of soybean 'NS 7007 IPRO' with similar germination percentages but different vigor were used. The seeds were supplied by the company Sementes Limoeiro, Patos de Minas, Minas Gerais state, Brazil. Initially. Seeds from each lot were classified based on differences in thickness (Vaughan et al., 1968) using a series of perforated metal screens with oblong (rectangular) openings varying by up to 0.4 mm (1/64″) in width. Seeds retained on the screen with dimensions 14 × 3/4″ ± 1/64″ (6.35 ± 0.4 mm), representing the medium seed size class (Pinto et al., 2018), were selected and subjected to various evaluations. To assess result consistency, the study was conducted across three evaluation periods, approximately three months apart. During this time, seed lots were stored in a dry cold chamber (10 °C and 30 % relative humidity) to minimize the progression of deterioration.

The following evaluations were performed in the laboratory on samples from each seed lot:

Seed water content: determined by the oven method at 105 ± 3 °C for 24 h (ISTA, 2022) on two samples of 5 g of seeds from each lot. Results were expressed as mean percentage (fresh weight basis) for each lot.

Germination: evaluated in four replicates of 50 seeds from each lot, distributed on moistened paper towel rolls with water equivalent to 2.5 times the dry paper weight, at 25 °C. Evaluations were carried out at four days (germination first count) and seven days after sowing and interpreted according to the International Rules for Seed Testing (ISTA, 2022).

Accelerated aging: plastic boxes (11 cm × 11 cm × 3.5 cm) were used as individual compartments (mini-chambers). The relative humidity within these boxes was controlled by adding 40 mL of water (creating an environment with approximately 100 % relative humidity) or a saturated sodium chloride solution to the bottom of each box. Seed samples from each lot were distributed in a single layer across the surface of a metallic screen suspended within each box. The boxes were then placed in a water-jacketed chamber maintained at 41 °C for 48 h (traditional accelerated aging - AA) or 72 h (saturated salt accelerated aging - SSAA). After these aging periods, germination tests were conducted at 25 °C for four days, as previously described. Results were expressed as the mean percentage of normal seedlings for each lot.

Electrical conductivity: four replicates of 50 seeds from each lot were weighed (0.01 g) and placed in plastic cups with 75 mL of deionized water. These samples were kept in a germinator at 25 °C for 24 h, and then the electrical conductivity of the solution was determined using a Digimed conductivity meter, model DM-32. Results were expressed in μS cm^{-1} g^{-1} of seeds.

Tetrazolium: three replicates comprising 50 seeds from each lot were preconditioned between sheets of moistened paper towels and maintained in a germinator at 25 °C for 16 h. Subsequently, the seeds were transferred to plastic cups containing 50 mL of a 0.075 % solution of 2,3,5-triphenyl tetrazolium chloride and incubated in an oven at 40 °C for 3 h for staining development. The percentage of seeds classified into viability classes 1 to 3 was recorded, and the results were expressed as the mean percentage for each lot.

Field seedling emergence: four replicates of 50 seeds were distributed in furrows 4 m long, 7 cm deep, and spaced 50 cm apart; seeds were covered with 3 cm of soil, and irrigation was provided as necessary to ensure adequate seedling emergence. The number of emerged seedlings was recorded daily to determine the speed of the seedling emergence index (Maguire, 1962). The seedlings that reached the vegetative stage cotyledon (cotyledons above the soil surface and unifoliate leaves with margins no longer touching) were computed. Fifteen days after sowing, the percentage of seedling emergence was calculated to obtain the mean values per lot.

Primary root emergence (RE): seed preconditioning was carried out using plastic boxes (11 cm × 11 cm × 3.5 cm) as individual compartments (mini chambers). Samples of each lot were distributed so as to constitute a single layer covering the entire surface of the metallic screen suspended within each box. The boxes were placed in a biochemical oxygen demand chamber set at 20 °C for 24 h. Subsequently, five replicates of 20 seeds each for each lot were distributed on two sheets of paper towel and covered with another, all moistened with a quantity of water equivalent to 2.5 times the mass of the dry paper. Samples were germinated for 24 and 30 h at 25 ± 1 °C or 30 ± 1 °C. Seedlings with a primary root of at least 1 mm in length (visual identification) were recorded and results were expressed as mean percentage for each lot.

Subsequently, images of seeds from each subsample were captured through transillumination. The roll containing the seeds, previously evaluated by visual analysis of RE, was directly positioned onto a platform consisting of a 40 × 40 cm LED panel with a power of 32 W

a) Perimeter: this corresponded to the determination of the two-dimensional seed contour, expressed in mm (Dell'Aquila, 2004b). An increase in perimeter value was associated to the RE.

b) Area: this represented the two-dimensional space (seed surface), expressed in square millimeters. An increase in the area value might be associated with RE (Oliveira et al., 2021).

c) Circularity: calculated by the formula

$$C = 4\pi \times \frac{[Area]}{[Perimeter]^2},$$

corresponding to the degree of approximation of the two-dimensional seed image to a circular shape (Ferreira

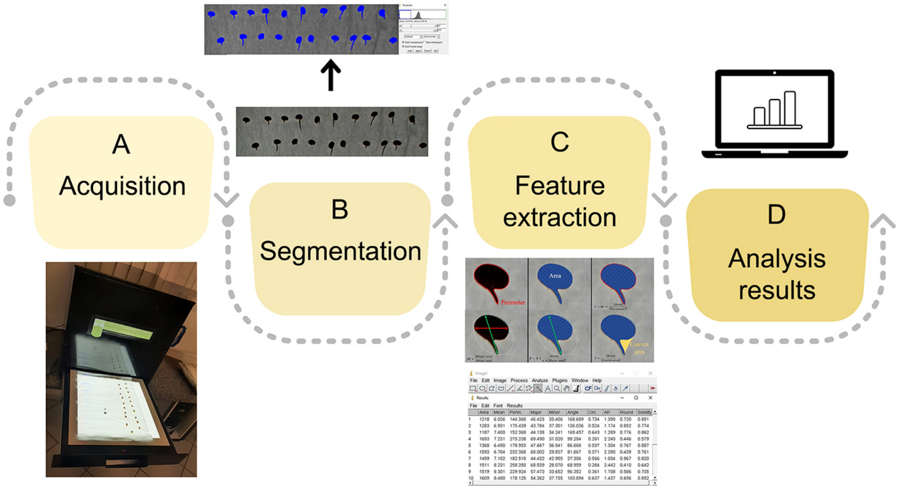


Figure 1 – Schematic representation of soybean seed image processing performed using ImageJ® software. The main procedures conducted are indicated in the boxes. A) Image acquisition and B) segmentation. C) Following segmentation, morphological descriptor extraction is performed, D) ultimately resulting in the generation of analyzable results.

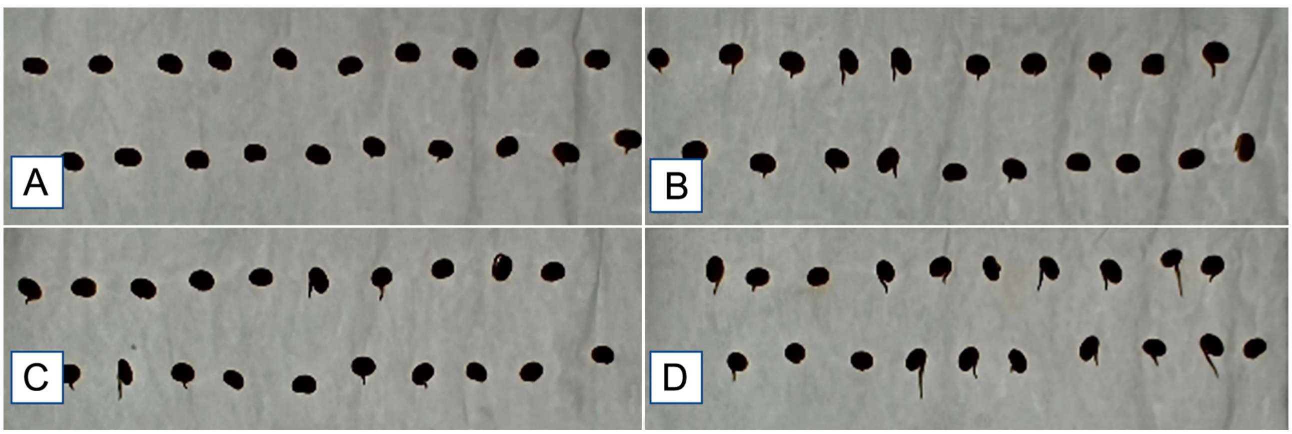


Figure 2 – Images of soybean seeds, cultivar NS 7007 IPRO, representing the treatments for seed lot evaluation based on primary root emergence. A) 24 h at 25 ± 1 °C, B) 30 h at 25 ± 1 °C, C) 24 h at 30 ± 1 °C, and D) 30 h at 30 ± 1 °C.

and Rasband, 2012). As a shape becomes less circular, circularity tends to approach zero. A value below one was associated with RE.

d) Aspect ratio: calculated using the formula

$$AR = \frac{[Major\ axis]}{[Minor\ axis]},$$

representing the ratio between the major and minor axes of the ellipse encompassing the seed (a greater ratio indicates how much more elongated the seed is). Reduction in these values might be associated with RE (Oliveira et al., 2021).

e) Roundness: calculated using the formula

$$R = 4 \times \frac{[Area]}{\pi \times [Major\ axis]^2},$$

representing the inverse of the aspect ratio, i.e., a lower ratio indicates a more circular seed (Dell'Aquila, 2004b);

f) Solidity: calculated using the formula

$$S \frac{[Area]}{[Convex\ area]},$$

representing the ratio of the projected area and the area of the convex hull (Ferreira and Rasband, 2012). A reduction in this value was associated with RE.

The results from the percentage of RE for each lot, obtained by visual interpretation and from morphological attributes determined using the ImageJ® software, were compared with the seed lot ranking based on the results of the recommended vigor tests performed here. This comparison aimed to establish criteria to identify their efficiency and potential use in composing quality control programs for soybean seeds.

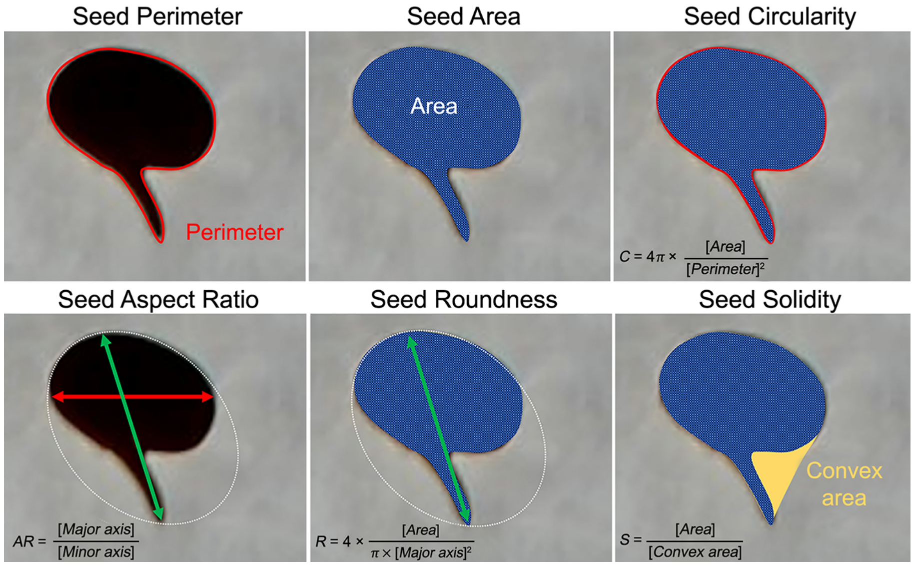


Figure 3 – Morphological descriptors used as indicators of changes potentially associated with the primary root emergence in soybeans.

Statistical procedure: the analysis of variance for the data from each test was carried out according to a completely randomized design, except for the field seedling emergence data, which were analyzed using a randomized block design. The data were tested for normal distribution of errors by the Shapiro-Wilk test and homogeneity of variances by the Bartlett test. Means were compared using the Scott-Knott test ($p < 0.05$). Data expressed as a percentage were transformed using the expression arc sen $(x/100)^{1/2}$ and those expressed in indices or centimeters were transformed using the expression $(x + 0.5)^{1/2}$. Statistical analysis was carried out using the SISVAR software (version 5.6). For the RE test, after image processing the results of the morphological parameters were saved in spreadsheets. The dataset was subjected to Principal Component Analysis (PCA) to reduce the dimensionality of the database and capture the most relevant information by establishing new orthogonal variables (factors) among themselves. These variables were used to verify the relationship between germination data, vigor, and morphological parameters obtained from soybean seed lots, considering different periods and temperatures during the RE test. The PCA was performed using the R software, version 4.0.0 (packages: tidyverse, stats and factoextra).

Results

Laboratory tests and primary root emergence

Results revealed that the germination in lots A7, A8, and A9 was lower ($p < 0.05$), as indicated by the Scott-Knott test (Table 1). The reduced vigor of seeds from these three lots was also detected by the germination first count, AA, and electrical conductivity tests; no differences were identified between the less vigorous lots (A4 to A9) by the tetrazolium and seedling emergence tests. In contrast, the highest vigor in lots A1, A2, and A3 was consistently identified by the results of all vigor and seedling emergence tests.

The seed lots submitted to the accelerated aging tests had an initial water content of 6.7 to 7.3 %. After the AA and SSAA periods, these values ranged between 21.9 and 24.6 %, and 11.7 and 12.3 %, respectively. These variations, below 2 to 3 %, are considered reliable for comparisons between samples tested for accelerated aging.

Results from RE evaluation are shown in Table 2. No differences in seed lot performance were detected when germinated at 25 °C for 24 h and at 30 °C for 30 h. However, when germination was carried out at 25 °C for 30 h and at 30 °C for 24 h, lots A1, A2, and A3 were identified as having higher physiological potential (Table 1).

Results from changes in morphological parameters as determined by the ImageJ® software are shown in Tables 3, 4, and 5. Significant variations were identified in seed perimeters depending on the germination conditions (Table 3). For instance, at 25 °C, both after 24 and 30 h, lots A1, A5, A8, and A9 exhibited the highest values, but when germination was conducted at 30 °C this pattern was not observed.

Variations in seed area were observed (Table 3), except for lots A8 and A9, which were detected in seeds exposed to all combinations of temperature and germination period. In general, there was similarity between the results of seed perimeter and area when germination was conducted at 25 °C for 24 h. However, changes in seed perimeter and area were not comparable to the ranking of seed lots established from vigor results, by RE and the other laboratory tests.

Table 1 – Average results of germination and vigor tests carried out in nine lots of soybean seeds, NS 7007 IPRO cultivar. Means from three evaluation times.

Lots	G	FC	AA	SSAA	EC	TZ-Vigor	FE	ESI
	%				µS cm^{-1} g^{-1}	%		index
A1	98 A*	98 A	85 A	93 A	69.3 B	94 A	81 A	8.72 A
A2	99 A	99 A	85 A	97 A	65.8 B	92 A	87 A	9.19 A
A3	97 A	96 A	88 A	98 A	63.6 B	93 A	88 A	9.04 A
A4	93 B	89 C	34 B	89 B	101.2 A	82 B	74 B	6.74 B
A5	95 A	87 C	22 C	87 B	108.0 A	85 B	62 B	5.37 B
A6	94 A	91 B	20 C	90 B	114.9 A	84 B	68 B	5.96 B
A7	92 B	84 C	12 D	89 B	113.9 A	83 B	63 B	5.49 B
A8	84 B	65 E	16 D	80 B	123.6 A	82 B	69 B	5.98 B
A9	89 B	76 D	12 D	86 B	120.5 A	82 B	67 B	5.90 B
CV (%)	4.75	6.25	12.62	4.86	9.91	3.81	13.42	13.19

*Means comparison within each column for each test (Scott-Knott test, $p < 0.05$). G = germination; FC = germination first count; AA = traditional accelerated aging; SSAA = saturated salt accelerated aging; EC = electrical conductivity; TZ-Vigor = tetrazolium test; FE = percentage of field seedling emergence; ESI = speed of seedling emergence index; CV = coefficient of variation.

Table 2 – Percentage of seedlings with primary root emergence obtained through visual evaluation after different germination periods and temperatures, in nine lots of soybean seeds, NS 7007 IPRO cultivar. Means from three evaluation times.

Lots	24 h at 25 °C	30 h at 25 °C	24 h at 30 °C	30 h at 30 °C
A1	25 A*	88 A	81 A	93 A
A2	31 A	83 A	81 A	88 A
A3	30 A	71 A	79 A	88 A
A4	35 A	57 B	62 B	86 A
A5	17 A	69 B	57 B	81 A
A6	27 A	65 B	64 B	93 A
A7	18 A	66 B	59 B	85 A
A8	32 A	66 B	56 B	86 A
A9	32 A	67 B	58 B	91 A
CV (%)	47.9	15.4	20.7	8.1

*Means comparison within each column (Scott-Knott test, $p < 0.05$). CV = coefficient of variation.

Evaluations of seed roundness showed that the superiority of lots A1, A2, and A3 performance was observed only when germination was conducted at 30 °C for 24 h (Table 4). This was also the case when determining the aspect ratio, when these lots produced lower values (Table 4), except in tests conducted at 30 °C for 30 h. A superior performance of lots A1, A2, and A3 in aspect ratio was also detected in roundness evaluations, and the same response was observed for the solidity parameter (Table 5) when germination was conducted at 30 °C for 24 h.

Relationships between morphological parameters and vigor assessments

Results from the three experimental periods showed differences in precision of the assessments of the parameters from morphological identification of RE by the ImageJ® software and its association with the seed vigor evaluations. As an example, images of one replicate (20 seeds/seedlings) and the values obtained for their respective perimeter, area, circularity, aspect ratio, roundness, and solidity, for each one of the seeds are shown in Table 6. It was observed that seeds 1 and 19 did not show RE but exhibited discrepancies in the area and perimeter results, i.e., 64.06 and 84.15 mm^2 in area and perimeter 30.47 and 35.33 mm, respectively. At the same time, in seed 4 there was RE, but the area value was the same as computed for seed 19, whereas for seed 12, where RE was also detected, the area was smaller than that determined for seed 19 (Table 6).

Table 3 – Seed perimeter and seed area determined by ImageJ® software after different germination periods and temperatures, in nine lots of soybean seeds, NS 7007 IPRO cultivar. Means from three evaluation times.

Lots	Seed Perimeter (mm)			
	24 h at 25 °C	30 h at 25 °C	24 h at 30 °C	30 h at 30 °C
A1	35.50 A*	38.51 A	39.65 A	54.87 A
A2	33.89 B	38.43 B	38.64 A	51.23 B
A3	32.10 C	36.22 B	37.40 B	48.25 C
A4	33.66 B	34.95 C	34.45 C	39.65 E
A5	35.53 A	36.75 B	37.16 B	41.91 D
A6	33.55 B	34.88 C	34.98 C	40.62 E
A7	33.07 B	34.46 C	34.61 C	38.43 E
A8	35.47 A	37.67 A	37.25 B	43.54 D
A9	35.17 A	37.70 A	36.95 B	43.33 D
CV (%)	1.90	2.18	3.10	4.51
	Seed Area (mm^2)			
A1	82.22 A	78.96 A	86.33 A	92.33 B
A2	74.88 B	78.96 A	78.64 B	86.16 C
A3	67.67 C	72.20 B	72.89 C	80.83 D
A4	75.18 B	75.45 B	74.27 C	79.26 D
A5	83.98 A	84.35 A	85.20 A	90.08 B
A6	74.53 B	73.65 B	74.93 C	80.40 D
A7	72.92 B	74.32 B	74.30 C	80.09 D
A8	82.89 A	87.19 A	84.97 A	93.79 A
A9	84.81 A	86.08 A	83.22 A	95.51 A
CV (%)	3.14	2.97	2.72	2.31

*Means comparison within each column (Scott-Knott test, $p < 0.05$). CV = coefficient of variation.

Table 4 – Seed circularity and aspect ratio determined by ImageJ® software after different germination periods and temperatures, in nine lots of soybean seeds, NS 7007 IPRO cultivar. Means from three evaluation times.

Lots	Seed Circularity (index)			
	24 h at 25 °C	30 h at 25 °C	24 h at 30 °C	30 h at 30 °C
A1	0.82 A*	0.75 B	0.71 A	0.43 A
A2	0.82 A	0.79 C	0.68 A	0.47 B
A3	0.82 A	0.71 A	0.68 A	0.50 B
A4	0.83 A	0.78 C	0.78 B	0.65 C
A5	0.83 A	0.79 C	0.78 B	0.66 C
A6	0.83 A	0.76 C	0.77 B	0.63 C
A7	0.83 A	0.79 C	0.78 B	0.69 C
A8	0.82 A	0.78 C	0.77 B	0.64 C
A9	0.81 A	0.76 C	0.77 B	0.65 C
CV (%)	1.70	2.71	3.56	6.29
	Seed Aspect Ratio (index)			
A1	1.43 A	1.33 A	1.32 A	1.57 B
A2	1.40 A	1.37 A	1.34 A	1.58 B
A3	1.44 A	1.33 A	1.35 A	1.53 B
A4	1.47 B	1.43 B	1.44 B	1.32 A
A5	1.50 B	1.47 C	1.46 B	1.36 A
A6	1.49 B	1.41 B	1.45 B	1.30 A
A7	1.50 B	1.46 C	1.46 B	1.35 A
A8	1.48 B	1.45 C	1.46 B	1.33 A
A9	1.50 B	1.46 C	1.47 B	1.34 A
CV (%)	2.13	2.15	2.56	4.61

*Means comparison within each column (Scott-Knott test, $p < 0.05$). CV = coefficient of variation.

Table 5 – Seed roundness and seed solidity determined by ImageJ® software after different germination periods and temperatures, in nine lots of soybean seeds, NS 7007 IPRO cultivar. Means from three evaluation times.

Lots	Seed Roundness (index)			
	24 h at 25 °C	30 h at 25 °C	24 h at 30 °C	30 h at 30 °C
A1	0.70 A*	0.74 A	0.76 A	0.67 B
A2	0.71 A	0.78 A	0.75 A	0.66 B
A3	0.69 A	0.75 A	0.74 A	0.69 B
A4	0.68 B	0.70 B	0.70 B	0.76 A
A5	0.67 B	0.68 C	0.68 B	0.74 A
A6	0.67 B	0.71 B	0.69 B	0.77 A
A7	0.66 B	0.68 C	0.69 B	0.74 A
A8	0.67 B	0.69 C	0.68 B	0.75 A
A9	0.66 B	0.68 C	0.68 B	0.75 A
CV (%)	2.12	2.01	2.61	3.82
	Seed Solidity (index)			
A1	0.95 A	0.91 B	0.89 A	0.73 A
A2	0.94 A	0.93 C	0.88 A	0.75 A
A3	0.94 A	0.89 A	0.88 A	0.78 A
A4	0.95 A	0.92 C	0.93 B	0.87 B
A5	0.95 A	0.93 C	0.93 B	0.88 B
A6	0.95 A	0.92 C	0.92 B	0.87 B
A7	0.95 A	0.93 C	0.93 B	0.89 B
A8	0.95 A	0.93 C	0.93 B	0.87 B
A9	0.95 A	0.92 C	0.93 B	0.87 B
CV (%)	0.67	1.14	1.40	2.60

*Means comparison within each column (Scott-Knott test, $p < 0.05$). CV = coefficient of variation.

As regards circularity, values below 0.70 were generally associated with RE, depending on the size reached (whether greater or smaller than 1.0 mm). For instance, in seeds 9 and 10, circularity values were 0.78 and 0.79, respectively, but only seed 9 exhibited RE (smaller than 1.0 mm). At the same time, in evaluations of the aspect ratio and roundness parameters, the seed shape showed an important influence, since aspect ratio values lower than 1.4 and roundness greater than 0.7 were associated with RE. With respect to the solidity parameter, lower values were detected in seeds with RE; in this case, values usually exceeded 0.95, and all seeds without emergence of the primary root (1, 2, 3, 8, 10, 11, 16, and 19) showed the same value (0.97). Overall, for all parameters, when the primary root size was less than 1 mm in length, the accuracy of the determinations decreased.

Through the PCA conducted on the dataset derived from the nine lots of soybean seeds exposed to germination under both periods (24 and 30 h) and two temperatures (25 and 30 °C) in three experimental periods, it was evident that the first two components (principal component = PC; PC1 and PC2) explain more than 90 % of the total data variability (Figure 4A-D). Under the interaction 25 °C and 24-h period, the most vigorous lots A1, A2, and A3 (Table 1) were positioned on the same side as the vectors corresponding to germination percentage, germination first count, AA and SSAA, tetrazolium, emergence of primary root, and roundness (Figure 4A).

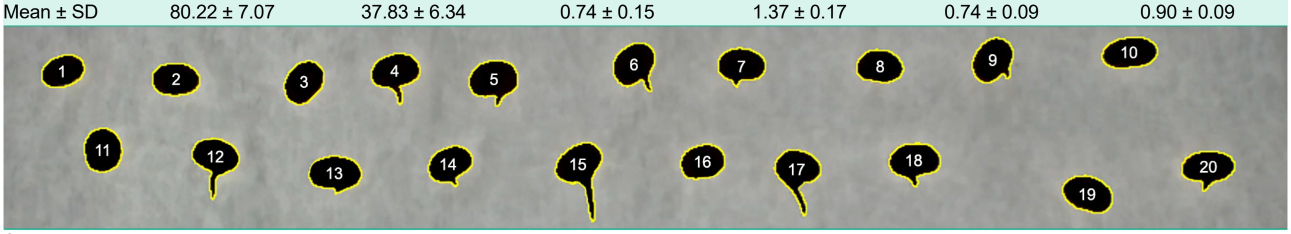
Similarities in vectors were observed between temperatures of 25 and 30 °C associated with the periods of 30 and 24 h, respectively (Figure 4B and C). For the temperatures and periods mentioned, the vectors of electrical conductivity, aspect ratio, circularity, and solidity were negatively related with the corresponding vectors of germination, germination first count, AA and SSAA, tetrazolium, percentage and speed of field seedling emergence, and RE (Figure 4B and C).

The temperature of 30 °C associated with the 30-h period resulted in changes in the orientation of vectors (Figure 4D). The vectors for perimeter, aspect ratio, and roundness shifted to a different quadrant, but they did not relate to the vectors from physiological potential results. The roundness vector shifts in relation to lots A4, A6, and A7 were considered less vigorous compared to lots A1, A2, and A3 (Table 1). This performance materializes a distinct association between roundness and the vectors of physiological potential evaluated under combinations of period and temperature during the RE test.

In general, the PCA analysis also showed that the area vector was not related to the seed lots' physiological potential results and morphological attributes, regardless of the period or temperature during the RE test (Figure 4A-D). This pattern might be related to what had previously been observed in Table 6, where the area value remained the same regardless of the presence or absence of RE in seeds 4 and 19.

Table 6 – Morphological parameters related to area, perimeter, circularity, aspect ratio, roundness, and solidity of a replicate of 20 seeds from a seed lot of NS 7007 IPRO cultivar.

Seeds	Area	Perimeter	Circularity	Aspect ratio	Roundness	Solidity
1	64.06	30.47	0.87	1.44	0.69	0.97
2	74.11	32.76	0.87	1.48	0.68	0.97
3	76.05	32.93	0.88	1.32	0.76	0.97
4	84.15	41.42	0.62	1.18	0.85	0.83
5	89.67	39.24	0.70	1.26	0.79	0.91
6	84.82	40.20	0.66	1.15	0.87	0.88
7	76.91	34.82	0.80	1.40	0.71	0.93
8	71.75	32.59	0.85	1.43	0.70	0.97
9	82.78	36.63	0.78	1.27	0.79	0.93
10	80.99	35.81	0.79	1.84	0.54	0.97
11	78.03	33.16	0.89	1.17	0.85	0.97
12	81.51	45.04	0.50	1.10	0.91	0.76
13	84.47	36.67	0.79	1.53	0.66	0.95
14	71.81	34.36	0.76	1.35	0.74	0.91
15	93.24	55.75	0.38	1.45	0.69	0.68
16	71.91	32.44	0.86	1.35	0.74	0.97
17	85.68	50.17	0.43	1.26	0.79	0.75
18	86.10	39.03	0.71	1.38	0.73	0.90
19	84.15	35.33	0.85	1.50	0.67	0.97
20	82.27	37.76	0.72	1.55	0.64	0.90
Mean ± SD	80.22 ± 7.07	37.83 ± 6.34	0.74 ± 0.15	1.37 ± 0.17	0.74 ± 0.09	0.90 ± 0.09



SD = standard deviation.

Discussion

The assessment of physiological potential (germination and vigor) in soybean seeds is an important component of "in house" quality control programs conducted by seed companies. Vigor results have not yet been included in marketing seed standards, despite playing a fundamental role in monitoring seed lot performance during storage and after sowing in the field by identifying relatively narrow variations in deterioration level among seed lots with similar germination (Baalbaki et al., 2009). Consequently, vigor tests must be simple, rapid, objective, efficient, inexpensive, and produce results to support the identification of problems and the development of proposals for solutions (Marcos-Filho, 2016).

Among the tests available, RE has gained prominence due to its efficiency, sensitivity, and rapidity in providing valuable information regarding the seed performance of different species (Matthews et al., 2010; Powell, 2022). The lack of RE test results for soybean seeds constituted the primary motivation for this research, along with the need to explore the possibility of employing computational resources as an alternative or enhancement to the procedure for assessing RE compared with results from recommended tests for assessing soybean seed vigor.

The nine seed lots utilized in the study possessed high germination (84 to 99 %), over the minimum requirements established for the commercialization of soybean seeds. This is of significant importance as seed companies are primarily interested in comparing the vigor of seed lots with acceptable germination levels (ISTA, 2022) and are favorably situated within Phase I of the survival curve (Powell, 1986). This also emphasizes the possible use of the results obtained in this study as a contribution when composing seed quality control programs.

Results indicated that lots A7, A8, and A9 exhibited significantly lower germination rates than the other lots. However, this test failed to accurately rank the lots with higher physiological potential. This finding is not surprising, as narrow differences in germinability, as observed in this study, usually increase the difficulty of detection by the germination test, as it is performed under optimal environmental conditions. In contrast, all the vigor and seedling emergence tests performed herein identified the significant superiority in the physiological potential of lots A1, A2, and A3, while lots A7, A8, and A9 were considered as lower vigor; these tests

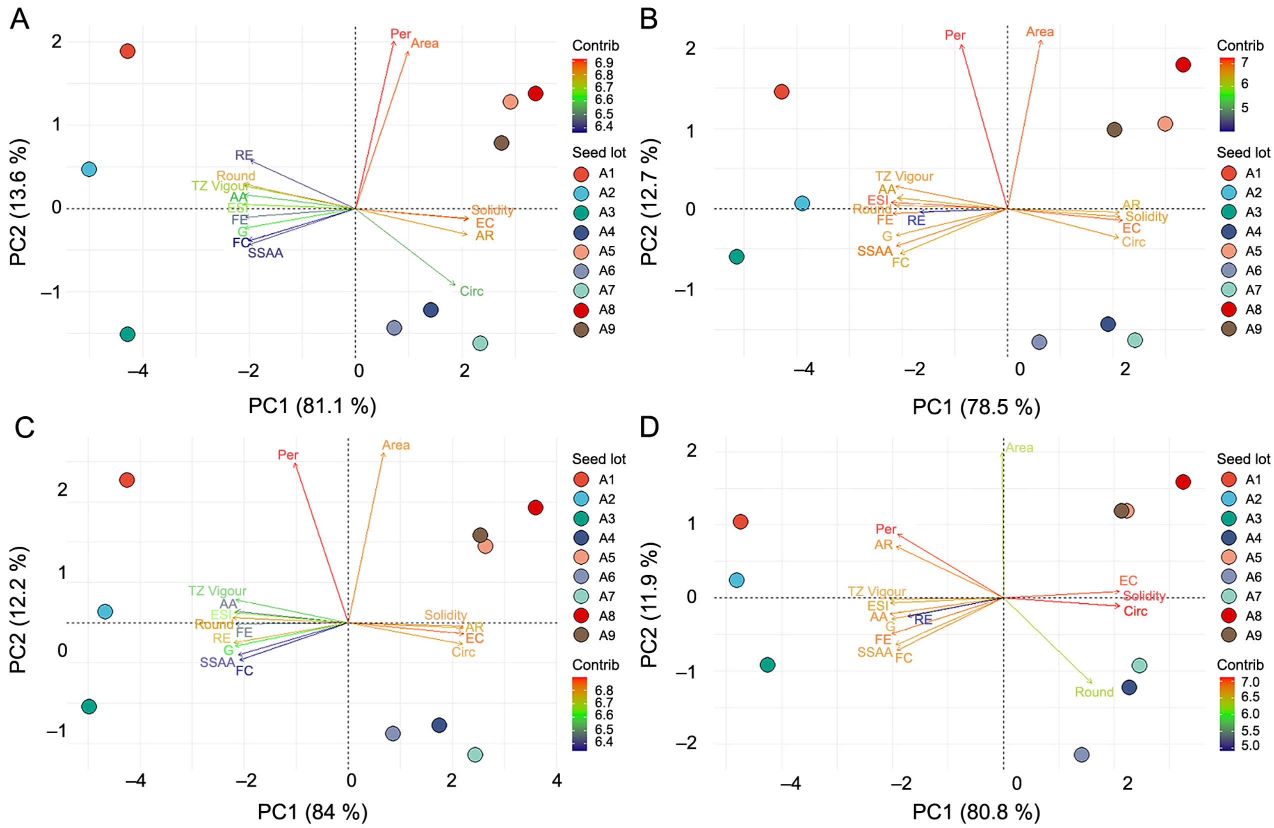


Figure 4 – Biplot graphs of principal component analysis comparing physiological potential assessments and morphological attributes determined by the ImageJ® software to characterize the primary root emergence of soybeans, cultivar NS 7007 IPRO, exposed to A) 24 h at 25 °C, B) 30 h at 25 °C, C) 24 h at 30 °C, and D) 30 h at 30 °C, in three experimental times. G = germination; FC = germination first count; AA = traditional accelerated aging; SSAA = saturated salt accelerated aging; EC = electrical conductivity; TZ-Vigor = tetrazolium test; FE = percentage of field seedling emergence; ESI = speed of seedling emergence index; RE = primary root emergence; Area = seed area; Per = seed perimeter; Circ = circularity; AR = aspect ratio; Round = roundness; PC1 = principal component 1; PC2 = principal component 2; Contrib = contribution.

demonstrated sensitivity to the detection of differences in seed lot performance. However, a number of tests showed no ability to discriminate differences between the less vigorous lots (A4 to A9). In this regard, the germination first count and the accelerated aging test were more efficient. As documented by the literature, it is crucial that vigor tests exhibit sensitivity sufficient to detect differences in vigor levels between lots with similar germination percentages. In addition, these tests must produce consistent results to differentiate high- and low-vigor seed lots and be able to associate laboratory and field seedling emergence or storability results.

On the other hand, the RE tests conducted at 25 °C for 24 h and 30 °C for 30 h could not distinguish the performance of different lots. However, when germination was carried out at 25 °C for 30 h and 30 °C for 24 h, the higher vigor of lots A1, A2, and A3 became evident. However, there was no consistent differentiation between the low-vigor lots (A4 to A9). Consequently, similar information between RE and that provided by vigor and seedling emergence tests was obtained concerning identifying the more vigorous seed lots. The speed of RE is associated with the period and amount of energy consumed for reactivating metabolism and repairing mechanisms during the germination process (Matthews and Khajeh-Hosseini, 2007; Matthews et al., 2010). Consequently, these events are less intense in deteriorated seeds, resulting in delayed germination and slower seedling growth rates. Therefore, higher percentages of seedlings with RE within a shorter period strongly indicate high seed vigor (Powell and Matthews, 2012).

As emphasized here, the efficiency in determining the speed of RE as a vigor test confirmed previous reports. This straightforward and time-effective method can be readily incorporated into seed quality assurance protocols. Previous studies have demonstrated its utility for evaluating cotton (*Gossypium hirsutum* L.) (Freitas et al., 2000), tomato (*Lycopersicon esculentum* Mill.) (Martins et al., 2006), and pepper (*Capsicum annuum* L.) (Demir et al., 2008) seeds.

Metabolic activity towards germination is resumed during imbibition at the same time as an increase in seed volume promotes the rupture of the seed coat and the primary root emerges (Taiz et al., 2017). These changes in seed size and shape are associated with RE; however, it is difficult to accurately characterize this event by visual analysis only. Therefore, the availability of computerized image analysis resources is often valuable for detecting variations in morphological parameters that enable the identification of RE and, consequently, its association with seed vigor (Dell'Aquila, 2007). As a result, the use of parameters from the ImageJ® software to detect the RE in germinated soybean seeds was incorporated into the project's design.

It was hypothesized that significant increases in seed perimeter and area should be directly related to the RE. However, it was observed that both parameters were not sufficiently sensitive to identify this occurrence, since the only consistent information provided by the perimeter determination was an emphasis on the means associated with lot A1, under all four germination conditions tested.

Simultaneously, there was an association between the perimeter and area results only in seeds germinated at 25 °C for 24 h (Table 3). Under other conditions, increases or decreases in perimeter were not accompanied by corresponding changes in area values and vice versa. In addition, the changes in perimeter and area values were not equivalent to the variations in vigor test results and visual evaluation of RE, indicating that the determination of both area and perimeter of soybean seeds was not efficient for characterizing RE or identifying the vigor level in the tested samples; this confirmed previous observations from Oliveira et al. (2021) with tomato seeds.

The parameters circularity, aspect ratio, roundness, and solidity were also studied by Dell'Aquila (2004b, 2009), Silva et al. (2013), Santos et al. (2020), and Oliveira et al. (2021) to associate seed morphological characteristics with germination and/or vigor. Our results demonstrated that circularity values below 0.7 were related to RE, but this occurred only when the primary root length exceeded 1.0 mm. There was no consistency of information when circularity exceeded 0.7 mm. For example, in seeds 5, 9, 13, 14, 18, and 20, the root length was less than 1.0 mm, but circularity values exceeded 0.7 (Table 6). Therefore, circularity values were generally associated with those obtained in vigor tests and the visual assessment of RE, mainly when germination was conducted at 30 °C for 24 h, confirming observations from Dell'Aquila (2004a) with cabbage seeds.

The determinations of aspect ratio (Table 4) and roundness (Table 5) also indicated the superiority of lots A1, A2, and A3, except when germination was instigated at 30 °C for 30 h. Aspect ratio values below 1.4, and roundness values above 0.7 were generally associated with RE. It is important to emphasize that this relationship occurred mainly when the length of the primary root was greater than 1.0 mm and that the information produced by these parameters was similar to those from vigor tests and visual assessment of RE.

Using the proposed methodology in this study, it took only one day to capture images of soybean seeds, plus a few minutes to process the images and obtain the results. This demonstrates that this research can potentially guide the development of plugins or macros for evaluating RE based on morphological parameters, using image processing by ImageJ®. Furthermore, macros specific to the seed technology field have already been found in the literature, created from ImageJ®, such as PhenoXray and IJCropSeed, which process radiographic images of seeds and correlate morphological parameters with their physiological potential (Medeiros et al., 2020a, b), and plugins like SeedsAnalyser, which identifies and analyzes multiple seeds represented in a digital image using morphological, texture, and color intensity parameters (Loddo et al., 2023).

The results observed after germination at 30 °C for 30 h may be associated with the fact that ImageJ® software interprets the circumscribed ellipse of the seed or seedling as an indication of RE (Table 6). Thus, when RE occurs, the circumscribed ellipse shows a major axis and a minor axis with a different proportion, accentuating the greater ratio between the ellipse's diameters. With exposure to higher temperatures over a longer germination period, root growth is evident, and the circumscribed ellipse becomes more elongated, resulting in a ratio between the ellipse's axes that is out of proportion. Furthermore, our study confirmed the utility of the aspect ratio as a reliable indicator of root emergence and seed vigor, as has been previously reported by Oliveira et al. (2021). The RE and the results of vigor tests were related to solidity values below 0.95, particularly when considering the length of the primary root (Table 6). This similarity in separating the more vigorous lots was associated with lower solidity values, as the growth of the primary root leads to an increase in the convex area, which is used as the denominator of the solidity equation (Figure 3). Therefore, vigorous seeds produce primary roots first, causing an increase in seed convexity, leading to lower solidity values. This means that more vigorous seeds tend to have lower solidity values.

Based on the results obtained in the three experimental periods and the biplot graphs from the PCA, the morphological parameters circularity, aspect ratio, roundness, and solidity, determined with the support of the ImageJ® software, demonstrated effectiveness in distinguishing lots according to vigor levels, particularly when germination was conducted at 30 °C for 24 h and considering the percentage of seeds that exhibited RE with a length over 1.0 mm. The criterion used to define the best moment for recording RE may differ slightly between species. However, it is, generally based on the identification of seeds that have produced seedlings with a minimum length of 2 mm (Powell, 2022). This demonstrates that a simple identification of the RE moment is sufficient for a consistent association with seed vigor. RE, evaluated using of computational tools, should be assessed when the root reaches a minimum length, which was established as 1.0 mm in the current case.

Thus, there was an indication of a direct relationship between the parameters obtained through the ImageJ® software, and the percentage of RE determined through visual analysis. This relationship was further associated with seed vigor assessed by tests recommended for soybean seeds. These parameters allowed for the identification of RE and its relationship with soybean seed vigor, thus establishing association with accepted vigor tests and being a rapid and objective option when compared with visual analysis for detecting RE. A computer-based image processing system is capable of measuring tomato seed germination with an accuracy of 95.44 %, showing the potential to be comparable to traditional germination and vigor tests performed in the laboratory (Škrubej et al., 2015).

Consequently, the utilization of image analysis resources represents a significant advance within the concept of "advanced technologies", particularly in terms of the speed and accuracy of seed and seedling analysis, as well as the reduction of human interference in the interpretation of results, especially when information is produced by different analysts. Considering the importance of soybeans for Brazilian and global agribusiness, more studies are required involving image analysis techniques on different cultivars to increase the efficiency of procedures for analyzing the vigor of seed lots.

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Conflict of interest

The authors declare having no conflict of interest.

Data availability statement

Not available.

Declaration of use of AI Technologies

The authors declare no use of AI Technologies.

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