Pesquisa Agropecuária Tropical
ISSN: 1517-6398
ISSN: 1983-4063
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Universidade Federal de Goiás
Brasil

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Pesquisa Agropecuária Tropical, vol. 48, núm. 3, 2018, pp. 222-228
Universidade Federal de Goiás
Goiânia, Brasil

e-ISSN 1983-4063 - *www.agro.ufg.br/pat* - Pesq. Agropec. Trop., Goiânia, v. 48, n. 3, p. 222-228, Jul./Sep. 2018

SAPL®: a free software for determining the physiological potential in soybean seeds[1]

André Dantas de Medeiros[2], Márcio Dias Pereira[3]

ABSTRACT

Obtaining image-based information is a powerful approach to capture and quantify seed vigor data. However, commercial systems that facilitate the processing and acquisition of images are often cost prohibitive. This study aimed to evaluate the efficiency of the Seedling Analysis System (Sistema de Análise de Plântulas - SAPL®), in order to analyze the physiological potential of soybean seeds, in comparison with the information provided by vigor tests which are traditionally recommended for this species. Nine lots of soybean seeds were submitted to germination, germination speed, seedling emergence, cold test and electrical conductivity tests. In the SAPL® analyzes, seedlings of four and six days, counted from the beginning of the germination test, were evaluated, resulting in values for seedling length, growth, development uniformity, vigor index and corrected vigor index. The evaluated lots with emergence greater than 90 % showed a vigor index higher than 600 and 800, respectively in the fourth and sixth days. The indexes generated by SAPL®, except for the uniformity index, presented positive and high correlations with the traditional tests (> 0.80). SAPL® is efficient in identifying differences in the vigor of soybean seed lots.

KEYWORDS: *Glycine max* (L.) Merill; physiological seed quality; seed technology.

RESUMO

SAPL®: um software gratuito para determinação do potencial fisiológico em sementes de soja

A obtenção de informações baseadas em imagens é uma abordagem poderosa para capturar e quantificar dados sobre o vigor de sementes. No entanto, sistemas comerciais que facilitam o processamento e a aquisição de imagens são, muitas vezes, de custo proibitivo. Objetivou-se avaliar a eficiência do Sistema de Análise de Plântulas (SAPL®), para analisar o potencial fisiológico de sementes de soja, em comparação com informações fornecidas por testes de vigor tradicionalmente recomendados para a espécie. Nove lotes de sementes de soja foram submetidos a avaliações de germinação, velocidade de germinação, emergência de plântulas, teste de frio e condutividade elétrica. Nas análises com o SAPL®, foram avaliadas plântulas com quatro e seis dias, contados a partir do início do teste de germinação, que resultaram em valores de comprimento de plântulas, crescimento, uniformidade de desenvolvimento, índice de vigor e índice de vigor corrigido. Os lotes avaliados com emergência superior a 90 % apresentaram índice de vigor superior a 600 e 800, no quarto e sexto dias, respectivamente. Os índices gerados pelo SAPL®, exceto o índice de uniformidade, apresentaram correlações positivas e altas com os testes tradicionais (> 0,80). O SAPL® é eficiente para identificar diferenças no vigor de lotes de sementes de soja.

PALAVRAS-CHAVE: *Glycine max* (L.) Merill; qualidade fisiológica de sementes; tecnologia de sementes.

INTRODUCTION

The use of computerized procedures capable of providing image-based information has been heralded as a powerful approach to capture and quantify data on physiological seed quality.

This type of procedure enables increasing the agility of the seed evaluation process, as well as providing a greater reliability in the obtained results (Marcos Filho 2015). The use of computerized analyzes to determine seed vigor has been successfully reported for several species, such as pumpkin (Silva et al. 2017), peanut (Barbosa et al. 2016), corn (Castan et al. 2018), wheat (Brunes et al. 2016), sunflower (Rocha et al. 2015), bean (Gomes Junior et al. 2014) and soybean (Marcos Filho et al. 2009, Wendt et al. 2014 and 2017, Yagushi et al. 2014), among others.

The commercial systems developed for this purpose are powerful tools for collecting image data and making inferences about seed vigor. However, commercial systems are cost-restrictive for many

1. Manuscript received in Apr./2018 and accepted for publication in Jul./2018 (http://dx.doi.org/10.1590/1983-40632018v4852340).
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laboratories, institutions and producers. To make high-performance computerized analysis methods more accessible, free software programs and low-cost equipment can be used.

The Seedling Analysis System (Sistema de Análise de Plântulas - SAPL®) appears as a free option for this type of analysis. SAPL® is based on the automated evaluation of seed vigor from seedling performance through digital image processing, which generates information about the length of the primary root, hypocotyl and total seedling length, as well as the root/hypocotyl ratio and parameters based on growth speed and uniformity. To do so, images obtained through photographs of the seedlings taken by smartphones or low-cost cameras are used. The software will soon be available for free download at the website of the Study Group on Seed Technology of the Universidade Federal do Rio Grande do Norte, along with a complete database.

This study aimed to evaluate the efficiency of SAPL® to analyze the physiological potential of soybean seeds, in comparison with the information provided by vigor tests which have been traditionally recommended for this species.

MATERIAL AND METHODS

The present research was conducted at the Universidade Federal do Rio Grande do Norte, in Macaíba, Rio Grande do Norte state, Brazil, in 2017. Nine lots of soybean seeds (BRS 7570 IPRO cultivar) from the region of Unaí (Minas Gerais state, Brazil) were evaluated through the following tests:

- Moisture content: determined before the germination and vigor tests, using the oven method at 105 ± 3 ºC, for 24 h (Brasil 2009), with two replicates for each lot. The results were expressed as mean percentage per lot;

- Germination test: conducted with four replicates of 50 seeds per lot, on paper rolls for seed germination moistened with distilled water in an amount equivalent to 2.5 times the dry paper weight. The rolls were packed into plastic bags and then kept in a BOD chamber at 25 ºC, for eight days. The evaluations were carried out in accordance with the criteria established in the Rules for Seed Analysis (Brasil 2009) and the results were expressed as mean percentage of normal seedlings for each lot;

- Seedling emergence: conducted with four replicates of 50 seeds, in expanded polystyrene trays seeded in individualized cells with washed sand substrate, and then kept in a greenhouse. The substrate was moistened whenever necessary and the final evaluation was carried out at 14 days after the test installation, when seedling emergence stabilized, expressing the result in percentage;

- Cold test: conducted with four replicates of 50 seeds, on rolls of paper moistened with an amount of distilled water equivalent to 2.5 times the weight of the substrate. After seeding, the rolls were placed inside plastic bags, sealed with adhesive tape and kept in a regulated chamber at 10 ºC, for five days. The choice of these days was based on pre-tests performed prior to the assembly of the experiment. After this period, the rolls were removed from the plastic bags and transferred to a germinator at 25 ºC, where they remained for four days, and then the number of normal seedlings was determined (Vieira & Krzyzanowski 1999);

- Germination speed: determined by means of the germination speed index together with the germination test, where daily counts of normal seedlings were performed at five to eight days after sowing, and calculated according to the formula proposed by Maguire (1962);

- Electrical conductivity: a total of four replicates of 50 seeds per lot were weighed in a scale with 0.001 g precision and placed in plastic cups containing 75 mL of distilled water, in germination chambers at 25 ºC, for 24 h. After this period, the electrical conductivity of the solution was determined in a TEC-4MP conductivity meter model, and the mean values were calculated and expressed in $\mu S\ cm^{-1}\ g^{-1}$ of seeds (Vieira & Krzyzanowski 1999);

- Analysis using SAPL®: four replicates of 20 soybean seeds per lot were distributed in two rows, in the longitudinal direction, on paper for germination previously moistened with distilled water in the amount equivalent to 2.5 times its dry mass. The seeds were positioned so that the hilum was facing the bottom of the paper, and the rolls were packed in plastic bags and placed vertically in the germinator, for periods of four and six days, at 25 ºC. At the end of each period, the seedlings were transferred from the paper towel to a blue (40 cm x 60 cm) satin vinyl foam sheet (EVA) containing nine cells (5 cm wide and 40 cm long), divided by white stripes. The top corner of the first cell on the right was dedicated to metrics (two points equidistant in 1 cm), and the rest of the cells were individually occupied by each seedling.

Images were acquired through photographs, using a DV300F Samsung digital camera. The images were then transferred to a computer and saved into folders identified for each repetition. The analysis was performed with the SAPL® software, installed on a HP Compaq Pro 6305 SFF computer, as shown in the sequence in Figure 1.

After analyzing and evaluating the seedlings, the software provided values related to the shoot, primary root and whole seedling length, as well as uniformity, growth, vigor and corrected vigor indices. These indices were defined by Sako et al. (2001), except for the corrected vigor index (CVI), introduced by the software developers based on the adaptive vigor index proposed by Sako et al. (2001), expressed by the following equation: $CVI = (0.70 * G + 0.30 * U) * (germ/100)$, where *CVI* is the corrected vigor index, *G* the growth index, *U* the uniformity index and *germ* the germination percentage.

The statistical analysis was performed in a completely randomized design with a unifactorial model, in which the factor lot contained nine levels with four replicates each. The data were submitted to analysis of variance (Anova). After confirming the normal distribution of errors by the Shapiro-Wilk test, the means were compared by the Scott-Knott test ($p < 0.05$). Data from traditional analyzes and SAPL® were also correlated by the Pearson's test (r; $p < 0.05$). The R statistical software was used in all analyzes (R Development Core Team 2014).

RESULTS AND DISCUSSION

Small differences were observed in the moisture content of the seeds, which varied between 8.5 % and 9.5 % (Table 1). This is considered a low variation and, according to Marcos Filho (2016), indicates that the seed lots can have their physiological quality compared to each other, since they are within the tolerable moisture limits for comparison, without being compromised by this factor.

For the germination test, it was observed that most of the lots presented a high viability, so that seven (lots 1, 2, 3, 4, 5, 8 and 9) of the nine evaluated lots presented values higher than 80 % for germination, being the minimum value established for commercialization of soybean seeds in Brazil (Brasil 2013). Lots 6 and 7 obtained germination percentages below 50 %, being considered as having a low physiological potential.

It is understood that significant differences in viability among lots are sufficient to infer about physiological quality disparities and, in this case, it is not necessary to complement them with vigor tests. However, this study aimed to use lots with different levels of physiological quality to verify the efficiency of the vigor evaluation methods, in this case making it important to contrast them.

Among the lots previously considered as having a high viability, the seedling emergence test classified the lots 1 and 3 as being superior, in relation to the others. Lots such as 2 and 9, with a previous germination equal to 90 %, presented a significant

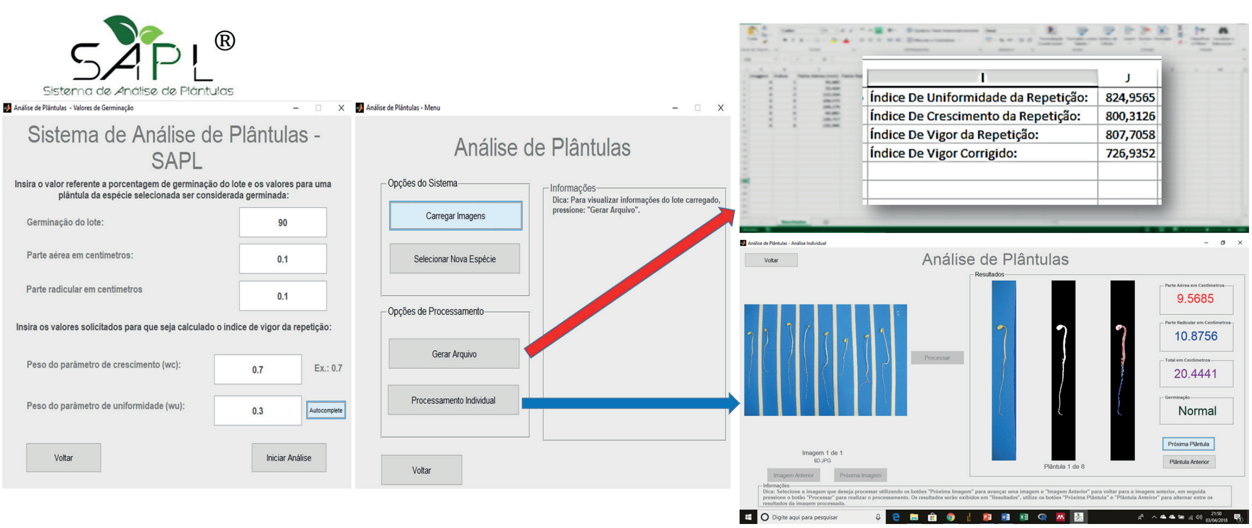

Figure 1. Representation of the image processing stages by the Seedling Analysis System (SAPL®).

Table 1. Mean results obtained in evaluating the physiological potential of soybean seeds through traditional tests.

Lot	Moisture degree	Germination	Emergence	Cold test	Electrical conductivity	Germination speed index
	%				µS cm^{-1} g^{-1}	
1	9.2	97.5 a*	93 a	82.5 b	63.5 e	33.2 b
2	9.0	90.0 b	68 c	67.0 c	96.7 d	25.7 c
3	8.5	99.5 a	94 a	95.0 a	57.4 f	36.4 a
4	9.2	96.5 a	80 b	81.5 b	63.7 e	26.1 c
5	8.5	89.0 b	76 b	51.0 d	123.8 c	26.9 c
6	9.5	44.5 d	31 d	29.0 e	127.6 b	09.1 e
7	9.2	66.0 c	22 e	17.0 f	149.3 a	12.1 d
8	9.5	93.5 b	81 b	78.5 b	61.6 e	28.3 c
9	9.0	90.0 b	79 b	78.0 b	65.1 e	24.7 c
CV (%)	-	4.7	6.9	11.9	2.3	7.8

* Averages followed by the same letter in the column do not differ by the Scott-Knott test ($p < 0.05$).

reduction in the number of normal seedlings, when submitted to the emergence test of 68 % and 79 %, respectively, indicating the low vigor of these lots. The emergence test seeks to identify vigorous seeds from conditions which are close to those observed at the field level, meaning uncontrolled conditions. In this sense, the information obtained from this test are valuable to predict the true physiological potential of the seeds.

Still in Table 1, it was possible to observe a close relation between the cold test and electrical conductivity. Both tests ranked the lots at six vigor levels, with the lot 3 achieving the highest performance in both tests. Moreover, significant differences were found among lots for the germination speed index, allowing a categorization into five vigor levels. As a priority, vigor tests seek to obtain complementary responses to those provided by the germination test (Ohlson et al. 2010), enabling the acquisition of reliable information to determine the seed physiological potential, but some adjustments related to standardization, precision and speed, in obtaining results, are still necessary (Silva et al. 2017).

In this research, the seedling image analysis generally allowed a greater agility in the evaluations, reducing the time spent to obtain the results. In the first evaluation performed on the fourth day after starting the germination test, physiological potential differences were identified among lots by means of seedling length (Table 2). Lot 3 was identified as having the highest length values; in contrast, lots 6 and 7 obtained lower growth rates. In evaluating the growth index, a grouping in seven vigor levels was verified, thereby allowing a more precise stratification of the lots, regarding physiological quality. The growth results corroborate those obtained by traditional physiological characterization tests for the lots tested in this study. This result is important, because one of the basic requirements for introducing new seed vigor evaluation procedures is their equivalence to other routinely used laboratory tests (Gomes Junior et al. 2014), or even in finding a greater sensitivity, in relation to the characterization of more vigorous lots.

There was no statistical difference for the uniformity variable between the tested lots in the two evaluated periods, since it is understood that, although they presented different lengths within the same lot, they maintained a homogeneous growth rate, which reflected in their non-differentiation, when the uniformity index was used. Studies by Caldeira et al. (2014), Chiquito et al. (2012) and Marchi & Cicero (2017) also did not demonstrate success in using the uniformity index for evaluating sunflower, cucumber or carrot seeds, respectively.

In the second evaluation performed on the sixth day after starting the germination test, it was observed that lots 1 and 3 (Table 2) presented a higher physiological potential than the others for seedling length and for all the evaluated indices. The analysis performed on the sixth day presented a lower stratification, if compared to the evaluation performed on the fourth day. This result may indicate that an earlier evaluation allows a greater refinement in the process of identifying quality differences among the tested lots, in addition to significantly reducing the time spent to perform the test. Researches with crambe (Leão-Araújo et al. 2017), pumpkin (Silva et al. 2017), bean (Gomes

Table 2. Mean results obtained by SAPL® in evaluating the physiological potential of nine lots of soybean seeds, at four and six days.

Lot	Seedling length (mm)	Growth index	Uniformity index	Vigor index	Corrected vigor index
		Four-day seedlings			
1	122 b*	485 b	885 a	605 b	590 b
2	70 e	265 f	894 a	454 d	408 b
3	152 a	609 a	886 a	692 a	689 a
4	108 c	412 d	865 a	548 c	529 c
5	73 e	281 f	838 a	448 d	398 e
6	47 f	156 g	826 a	357 e	158 g
7	44 f	164 g	838 a	366 e	240 f
8	108 c	440 c	837 a	559 c	522 c
9	84 d	366 e	880 a	520 c	468 d
CV (%)	7.7	7.2	12.2	6.2	5.1
		Six-day seedlings			
1	237 a	934 a	785 a	889 a	867 a
2	167 c	629 c	716 a	655 c	590 c
3	238 a	935 a	751 a	880 a	876 a
4	212 b	859 b	707 a	813 b	785 b
5	161 c	658 c	748 a	685 c	610 c
6	111 d	432 d	702 a	513 d	227 e
7	103 d	368 d	782 a	492 d	324 d
8	202 b	824 b	778 a	810 b	758 b
9	168 c	673 c	761 a	699 c	629 c
CV (%)	7.2	8.5	8.0	5.8	7.1

* Means followed by the same letter in the column do not differ by the Scott-Knott test ($p < 0.05$).

Junior et al. 2014) and soybean seeds (Marcos Filho et al. 2009, Wendt et al. 2014 and 2017), among other crops, have also demonstrated promising results in earlier evaluations.

Regarding the use of the vigor index and corrected vigor index for the two evaluation periods (Table 2), both were able to rank the lots for their physiological quality. The corrected vigor was able to achieve promising results, showing itself to be sensitive for categorizing the lots for vigor. Corrected vigor is an index made up of the product of the vigor index with the germination percentage, and thus a more efficient beaconing of the vigor results is obtained. According to Nakagawa et al. (1999), this adjustment is important, because, in order to correctly perform a lot quality evaluation together with the results obtained in the seedling length test, the germination percentage should be taken into account, since there are cases in which the lot presents a low germination percentage and a high average seedling length value, or the opposite. In this situation, the few normal seedlings formed (low germination percentage) present a high growth rate, a fact that cannot be transposed for the whole sample or the whole lot, in considering it to be vigorous. Therefore, it is necessary to take into account the length and uniformity of the seedlings in the lot in question, thereby also having the germination value as a parameter.

From the correlation analysis presented in Table 3, the limitation of the uniformity index

Table 3. Pearson's correlation (r) among the variables generated from the traditional seed analysis tests and the digital image processing with SAPL® of seedlings from nine lots of soybean seeds.

SAPL®	Traditional tests				
	G (%)	E (%)	CT (%)	GSI	EC (μS cm^{-1} g^{-1})
			Four-day seedlings		
SL (mm)	0.79*	0.87*	0.85*	0.89*	-0.84*
Gr	0.81*	0.88*	0.86*	0.90*	-0.87*
U	0.66^{ns}	0.62^{ns}	0.69*	0.65^{ns}	-0.62^{ns}
V	0.82*	0.89*	0.88*	0.91*	-0.88*
CVI	0.92*	0.93*	0.90*	0.96*	-0.90*
			Six-day seedlings		
SL (mm)	0.86*	0.93*	0.93*	0.93*	-0.87*
Gr	0.86*	0.94*	0.93*	0.92*	-0.86*
U	0.30^{ns}	0.17^{ns}	0.14^{ns}	0.28^{ns}	-0.32^{ns}
V	0.87*	0.94*	0.93*	0.93*	-0.87*
CVI	0.94*	0.95*	0.93*	0.96*	-0.89*

*, ^{ns}: significant and not significant, respectively, at a 5 % probability. G: germination, E: emergence; CT: cold test; GSI: germination speed index; EC: electrical conductivity; SL: seedling length; Gr: growth; U: uniformity; V: vigor; CVI: corrected vigor index.

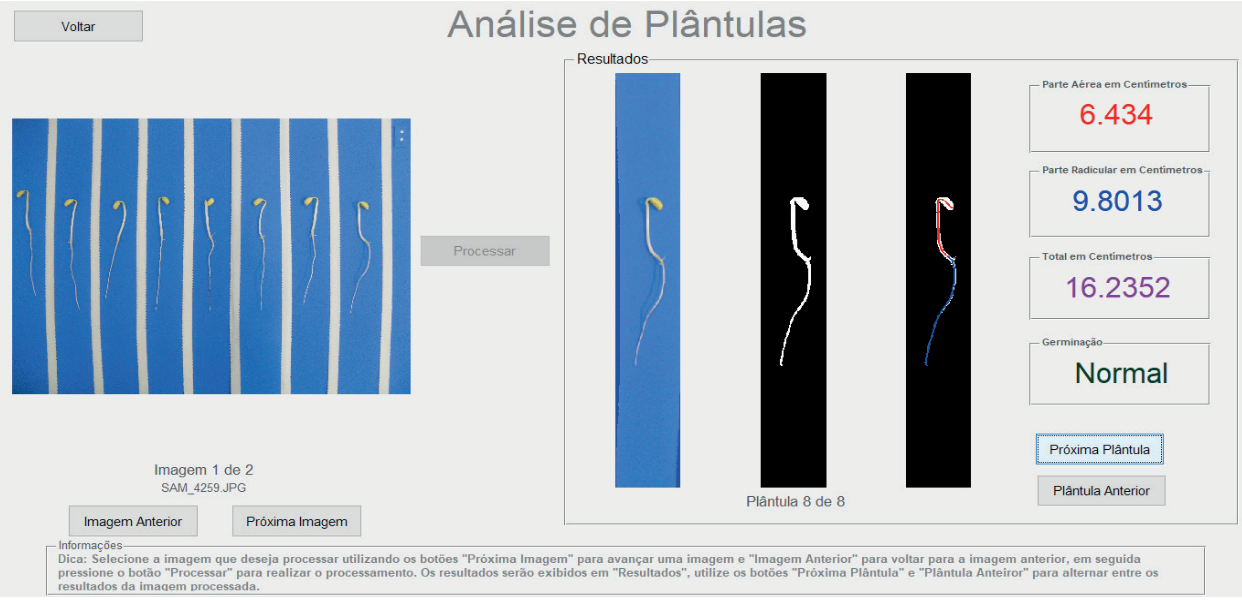

Figure 2. SAPL® individual seedling processing screen, in which four-day soybean seedlings are being evaluated for their length.

is evident for classifying the lots and to express a univocal relation with other variables. Thus, this index should not be considered in the digital physiological quality analysis of the seeds. The other variables generated from SAPL® showed a high correlation with the traditional tests, indicating a close relationship between them. According to Albuquerque et al. (2008), the number of tests evaluated in future experiments may be reduced with linear correlation analysis, as a great dependence between the variables is observed.

One of the difficulties to determine the seedling length is the tendency for a hypocotyl curvature of the seedlings (Gomes Junior et al. 2014). However, automating these determinations significantly contributes to reduce errors made by analysts in seed laboratories, who routinely work intensively at evaluating a high number of replicates. Figure 2 shows the print screen of the individual seedling processing window in SAPL®, in which the shape of each seedling is evaluated.

Important information about the growth potential and uniformity of seed germination are fundamental pillars for successfully establishing plants in the field. Thus, the results from this study are promising for evaluating the quality of soybean seeds via the SAPL® software and, based on this research, it could easily be included in the analyzes performed by laboratories, institutions and by rural producers themselves. According to Gomes Junior et al. (2014), in addition to the consistency of results, the analysis of seedling images to obtain lot vigor contributes to the information management and to store images and numerical results for future studies.

CONCLUSIONS

1. The Seedling Analysis System (Sistema de Análise de Plântulas - SAPL®) is efficient to identify differences in the vigor of soybean seed lots, if compared to tests traditionally used for the species;
2. The analysis performed on four-day seedlings proved to be more appropriate, since it enables a better ranking of seed lots and a greater precocity in obtaining the results.

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