ABRATES

Journal of Seed Science
ISSN 2317-1545
www.abrates.org.br/revista

ARTICLE

Vigor-S: System for Automated Analysis of Soybean Seed Vigor

Journal of Seed Science, v.42, e202042039, 2020

http://dx.doi.org/10.1590/2317-1545v42237490

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ABSTRACT: Computerized systems for image analysis are alternatives to decrease the subjectivity and speed of assessment of seed physiological potential. The aim of this study was to determine the efficiency of the Seed Vigor Automated Analysis System (Vigor-S) to identify differences in vigor among soybean seed lots compared to results of the Seed Vigor Imaging System – SVIS® and tests recommended for evaluation of soybean seed vigor. Two cultivars were used, BMX Potência RR and 7166 RSF IPRO, each one represented by ten seed lots with similar germination and vigor differences. The seeds were evaluated regarding germination, vigor (tetrazolium, accelerated aging), seedling emergence in the field and SVIS® and Vigor-S analysis, in three experimental periods. Analysis of variance was used on the data in a completely randomized experimental design (laboratory tests) and in randomized blocks (field tests), and the mean values were compared by the Tukey test ($p \leq 0.05$). Separation of the seed lots by Vigor-S was consistent with the results obtained in tests recognized as effective in evaluating the physiological potential of soybean seeds, and there was no interference of the different cultivars on the response pattern for vigor. Analysis through Vigor-S proved to be effective in determination of physiological potential and for composition of quality control programs established by soybean seeds production companies.

Index terms: *Glycine max*, SVIS®, quality control, physiological potential.

Vigor-S: Sistema para Avaliação Automatizada do Vigor de Sementes de Soja

RESUMO: Sistemas computadorizados de análise de imagens são alternativas para diminuir a subjetividade e o tempo de avaliação do potencial fisiológico de sementes. O objetivo desta pesquisa foi verificar a eficiência do sistema de Análise Automatizada do Vigor de Sementes (Vigor-S) para identificar diferenças de vigor entre lotes de sementes de soja, em comparação aos resultados do Seed Vigor Imaging System – SVIS® e testes recomendados para avaliação do vigor de sementes de soja. Utilizaram-se duas cultivares, BMX Potência RR e 7166 RSF IPRO, cada uma representada por dez lotes de sementes com germinação semelhante e diferenças de vigor. Foram realizados testes de germinação, vigor (tetrazólio, envelhecimento acelerado), emergência de plântulas em campo e análises SVIS® e Vigor-S, em três épocas experimentais. Os dados foram submetidos a análise da variância, em delineamento inteiramente casualizado (testes em laboratório) e em blocos casualizados (teste em campo), com médias comparadas pelo teste de Tukey ($p \leq 0{,}05$). A separação de lotes pelo Vigor-S foi coerente com os resultados dos testes reconhecidamente eficientes para avaliar potencial fisiológico de sementes de soja, sem interferência das cultivares no padrão de resposta às diferenças de vigor. A análise pelo sistema Vigor-S é eficiente para determinação do potencial fisiológico e composição de programas de controle de qualidade estabelecidos por empresas produtoras de sementes de soja.

Termos para indexação: *Glycine max*, SVIS®, controle de qualidade, potencial fisiológico.

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Received: 5/2/2020.
Accepted: 9/14/2020.

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INTRODUCTION

Evaluation of seed physiological potential is a fundamental component of quality control programs conducted by seed production companies. Although the results of vigor tests are not components of the standards established for the seed trade in Brazil or internationally, they are very important for evaluating the performance of seed lots during storage and after sowing in the field. They are able to detect relatively small variations in the stage of deterioration of seed lots with similar germination percentages (Baalbaki et al., 2009), constituting powerful aids in making decisions regarding how the lots are handled. However, as seed vigor gathers a set of characteristics, adoption of only one test, whether physiological, biochemical, or of stress resistance, generates incomplete information because a single test is not sufficient to accurately identify the performance potential of seeds exposed to varied environmental conditions (Marcos-Filho, 2015a).

Tests that evaluate seedling development are recognized and recommended both by international associations dedicated to the development of tests for seed quality assessment (ISTA – International Seed Testing Association and AOSA – Association of Official Seed Analysts) and by the Brazilian seed technology association (ABRATES – *Associação Brasileira de Tecnologia de Sementes*). Nevertheless, if the determinations of the length of the total seedling or of its parts are performed according to the traditional procedure (using a ruler), the time required is excessive and results are imprecise, generally giving rise to results that vary from one laboratory or analyst to another. To minimize limitations regarding possible deficiencies of standardization in vigor tests, procedures with the assistance of computerized resources have been developed as an alternative that not only speeds the process of obtaining consistent information but also leads to more precise results (Marcos-Filho, 2015b).

The Vigor-S (Seed Vigor Automated Analysis System) is a system developed by Brazilian researchers of the *Universidade de São Paulo* (USP/ESALQ) and of the *Embrapa / Instrumentação Agropecuária* (Crop and Livestock Instrumentation Service) for evaluation of seed vigor based on seedling performance. The principle of this system is the same that underlies the SVIS® (Seed Vigor Imaging System), developed by The Ohio State University, which proved to be promising but which has limitations for use and for enhancement of procedures due to characteristics of the patent.

Just as the SVIS®, the Vigor-S produces information regarding vigor indices, uniformity of development, and mean seedling length. The two systems evaluate the vigor index based on the rapidity and uniformity of seedling development of the sample in relation to the maximum value estimated for seedlings of an age pre-established on the software program. They exhibit advantages regarding fast results, reduction in human influence (increasing the reliability of the data), and the possibility of filing the images for later analysis. Unlike the SVIS®, the Vigor-S for evaluation of the physiological potential of soybean seeds and of species whose seedlings have a similar skeleton is available at https://drive.google.com/open?id=0B8rVh_veepVzdXM0MlJ1M0I0ZEk, with free access by internet, which makes its use feasible for all who have interest.

In light of the above, the aim of this study was to check the efficiency of the Seed Vigor Automated Analysis System (Vigor-S) in identification of vigor differences in soybean seed lots in comparison to the Seed Vigor Imaging System – SVIS® and other tests recommended for evaluation of soybean seed vigor.

MATERIAL AND METHODS

The study was conducted at the *Escola Superior de Agricultura Luiz de Queiroz*, *Universidade de São Paulo* (USP/ESALQ), in Piracicaba, SP, Brazil. Two soybean cultivars were used, BMX Potência RR and 7166 RSF IPRO, each one represented by ten seed lots not subjected to chemical treatment, with similar germination percentages but with vigor differences. The seeds remained in paper bags in cold and dry storage (10 °C and 30% relative humidity) throughout the experimental period. The study was conducted on seeds retained in metallic sheet sieves with oblong holes between 15 × 3/4’’ (6.0 × 19.0 mm) and 17 × 3/4 (7.0 × 19.0 mm), defined as based on preliminary information (Pinto et al., 2018).

The study was conducted in three experimental periods, maintaining the seeds under the same climate-controlled conditions (10 °C and 35% RH), with intervals of approximately three months, with the aim of checking the consistency of the results. The following evaluations were performed in the laboratory on samples from each seed lot:

Moisture content: This was determined by the laboratory oven method at 105 ± 3 °C for 24 hours (Brasil, 2009) on two 5 g samples of seeds from each lot. Moisture content was also determined after seeds were exposed to the accelerated aging test.

Germination: This was evaluated in four replications of 50 seeds each in rolls of paper towel moistened with water in the amount of 2.5 times the dry weight of the paper, at 25 °C. The evaluations were carried out at four days (first germination count) and at seven days after sowing; the test was interpreted according to the criteria established in the Rules for Seed Testing (Brasil, 2009).

Accelerated aging: Plastic boxes (11 cm × 11 cm × 3.5 cm) were used as individual compartments. The relative humidity within these boxes was the result of addition of 40 mL of water (environment with approximately 100% RH) or of saturated sodium chloride solution (environment with 76% RH) at the bottom of each box (Marcos-Filho et al., 2000). The 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. Accelerated aging was conducted at 41 °C for 48 hours (traditional accelerated aging test – TAA) or 72 hours (saturated NaCl solution accelerated aging test – SSAA) in a water-jacketed chamber. In both situations, this was followed by the germination test at 25 °C over four days. The mean percentage of normal seedlings for each lot and cultivar was computed (Marcos-Filho et al., 2000).

Tetrazolium: 150 seeds were used (three subsamples with 50 seeds each, for each lot and cultivar). The seeds were pre-conditioned between sheets of paper towel, previously moistened with water in the amount of 2.5 times the weight of the dry paper, and they were then kept in a seed germinator at 25 °C for 16 hours. After that, the samples were placed in plastic cups containing 50 mL of a solution of 2,3,5 triphenyltetrazolium chloride at 0.075% and kept in a laboratory oven at 40 °C for three hours for development of coloring (França-Neto and Krzyzanowski, 2018). At the end of this period, the seeds were washed in running water and remained submerged in water in a cooled environment at 5 °C up to the time of evaluation. The test was interpreted based on seed classification adapted by França-Neto and Krzyzanowski (2018), where each seed is placed in classes from 1 to 5, where viable, and from 6 to 8 if not viable; the level of viability is identified according to an order of increasing "defects" of the seeds. The vigor index considers the percentages of seeds included in classes 1 to 3.

Seedling emergence in the field: Four replications of 50 seeds each were used, distributed at equal distance in furrows of 4 m length with 40 cm distance between furrows. The area was irrigated by a sprinkler system, with a water depth of 7 $mm.day^{-1}$ to ensure adequate emergence of the seedlings. The number of emerged seedlings was recorded daily to determine the emergence speed index (Maguire, 1962). The seedlings that reached the VC stage were counted, that is, seedlings with cotyledons above the soil surface and unifoliate leaves with the leaf margins no longer touching, as described in Costa and Marchezan (1982). At approximately 15 days after sowing, when seedling emergence stabilized, the emergence percentage was calculated, obtaining the mean values per lot and cultivar.

Computerized seedling image analysis (SVIS® and Vigor-S): Five replications of 20 seeds each were used per lot of each cultivar distributed on two sheets of paper towel (two rows of 10 seeds each) and covered with a third sheet. The substrate was previously moistened with water in the amount of 2.5 times its dry weight.

For use of the Vigor-S software, the rolls containing the seeds were kept in a seed germinator at 25 °C for three days. At the end of that period, the seedlings from each replication were transferred from the roll of paper towel to a sheet of EVA (ethylene vinyl acetate) of blue color, with dimensions of 30 cm × 22 cm (corresponding to the size of the effective area of the scanner) to provide the necessary contrast for analysis by the system. After that, the seedling images were digitalized using a HP Scanjet 200 scanner set up in an inverted position within an aluminum box (60 × 50 × 12 cm), adjusted to a resolution of 300 dpi and connected to a Core i7 computer (3.50 GHz and 16 GB of RAM).

The images were processed by the software, which records the pre-established maximum length of seedlings

(12.7 cm for soybean after preliminary tests) in its operating system. This value cannot be changed by the user; it is used to calculate the growth index. Thus, the mean values of the vigor index were obtained, encompassing values from 0 to 1000, as well as the mean length of the hypocotyl, the primary root, and the total of the seedlings (Figure 1); seed vigor is directly proportional to these values.

According to the framework parameter of the systems, the contribution of the value of growth and of uniformity to calculation of the vigor index was 70% and 30%, respectively, values which were likewise established after preliminary tests. The equations to obtain the growth uniformity index and vigor index were established based on the equations of the SVIS® system. For the uniformity index equation, an adaptation of Christiansen's uniformity coefficient (Christiansen, 1942) was made, which was presented by Castan et al. (2018). Furthermore, for the Vigor-S system, the contribution of the value of the hypocotyl and of the primary root to the calculation of seedling growth was 10% and 90%, respectively. This value can be changed, but it is important that it be established for all the samples. The steps of seedling image assessment by the Vigor-S system can be found in Castan et al. (2018), who worked with maize seeds.

To obtain results by the Seed Vigor Imaging System (SVIS®), the images with resolution of 300 dpi used in the Vigor-S system were transformed to 100 dpi through the Adobe Photoshop® program, and analyzed by the SVIS®. The maximum size of the seedlings was fixed at five inches (12.7 cm). Thus, the mean indices of vigor and the mean length of seedlings were generated for each lot (Marcos-Filho et al., 2006). The contribution of the growth value and uniformity value to calculation of the vigor index was also 70% and 30%, respectively.

At the end of the three periods of evaluation, the data of each test were clustered and analyzed. Normality of the residues was checked by the Shapiro-Wilk test and homogeneity of the variances. When necessary, the data expressed in percentage were transformed in arcsine $(x/100)^{1/2}$ and those expressed in indices or centimeters were transformed in $(x + 0.5)^{1/2}$. Analysis of variance for the data in reference to each cultivar and test was conducted according to a completely randomized design, except for the data of seedling emergence, which were analyzed according to the randomized block design. Statistical analysis was performed using the AgroEstat software (Barbosa and Maldonado-Junior, 2010) and mean values were compared by the Tukey test ($p \leq 0.05$).

Figure 1. General layout of the analysis screen of the Vigor-S software after processing of seedling images.

RESULTS AND DISCUSSION

BMX Potência RR Cultivar (Cultivar 1)

Evaluation of seed physiological potential

Analysis of variance of the data indicated significant F values for effects of lots in all the tests performed. The mean values obtained in the germination and vigor tests (tetrazolium, accelerated aging, and seedling emergence) and the respective coefficients of variation are shown in Table 1.

The results obtained in the germination test identified significant superiority of lots L6, L7, L8, and L10 in relation to the other lots, which did not differ from each other. It should be emphasized that all the lots exceeded the minimum germination established for the soybean seed trade in Brazil (G ≥ 80%). This is important because it would not be consistent to evaluate the vigor of soybean seed lots with germination lower than 80%, that is, evaluation of seeds not suitable for sowing in commercial fields.

The data of the tetrazolium test (classes 1 to 3) confirmed the greater vigor of L7, not differing from L10; at the same time, L9 was identified as having lowest vigor, not differing from L2 and L5. França-Neto and Krzyzanowski (2018) considered that values higher than 85% in the tetrazolium-vigor test (classes 1-3), characterize lots as very high vigor; from 84% to 75% as high vigor. Values lower than 59% correspond to lots with low or very low vigor. Thus, in this study, lots with low or very low vigor were not identified, because L2, L5, and L9, identified as those of lowest vigor, had values of 66%, 65%, and 61%, respectively.

In regard to the results obtained in the traditional accelerated aging test (TAA), lots L7 and L8 were identified as superior to the others. Just as in the tetrazolium test, lots L5 and L9 were those of lowest physiological potential. Comparing the accelerated aging data with those of germination, L7 and L8 maintained the percentage of normal seedlings nearest to that obtained in the germination test. This indicates that, theoretically, these lots have greater tolerance to adverse situations (Marcos-Filho, 2015a). Seeds of low physiological potential deteriorate more rapidly than more vigorous ones in the traditional accelerated aging test, with an accentuated decline in viability (Baalbaki et al., 2009), which was observed for lots L5 and L9.

Table 1. Germination, tetrazolium-vigor, traditional accelerated aging (TAA 48 h) and accelerated aging with saturated NaCl solution (SSAA 72 h), seedling emergence percentage and speed in 10 soybean seed lots, cultivar BMX Potência RR (Cultivar 1). Mean of three experimental periods.

Lot	G	TZ – Vigor	TAA 48 h	SSAA 72 h	Seedling emergence	
			%		%	Index
L1	87 b	86 bc	65 b	79 cd	76 bc	4.36 ab
L2	87 b	66 ef	35 c	75 cd	62 d	3.41 cd
L3	87 b	71 de	30 c	73 d	69 cd	3.81 bc
L4	91 b	74 d	36 c	77 cd	70 cd	3.82 bc
L5	88 b	65 ef	14 d	49 e	59 d	3.08 d
L6	97 a	84 c	64 b	90 b	80 ab	4.50 ab
L7	98 a	94 a	87 a	97 a	88 a	5.05 a
L8	96 a	89 bc	89 a	95 ab	88 a	5.00 a
L9	86 b	61 f	7 e	51 e	59 d	3.05 d
L10	96 a	90 ab	70 b	81 c	85 ab	4.85 a
CV(%)	6.2	4.3	8.6	7.3	9.1	6.0

Lowercase letters: comparisons among means within each column, in separation for each test conducted (Tukey Test, p ≤ 0.05).

Similar information regarding separation of lots into different vigor levels was provided by the accelerated aging test with saturated NaCl solution (for 72 hours), once more with prominence of L7, which did not differ from L6 and L8. Lots L5 and L9 were least vigorous.

In spite of the similar results regarding separation of the lots, the reduction in seed vigor brought about by the conditions of the traditional accelerated aging test was more accentuated than in the SSAA. This occurs because the relative humidity within the plastic boxes with saturated NaCl solution (76%) is lower than that maintained in the traditional test (near 100%), allowing uniform reduction in the speed of water absorption by the seeds, the intensity of deterioration, and the degree of stress placed on the seeds. The effectiveness of the procedure with use of a saturated sodium chloride solution for evaluation of soybean seed vigor was investigated by Marcos-Filho et al. (2000), Marcos-Filho et al. (2009), and Yagushi et al. (2014).

The variation in moisture content of the seeds of the cultivar BMX Potência RR at the beginning of the evaluations was from 7.7% to 10.0%. After the period of TAA, the variation was from 24.1% to 27.4%, and after the SSAA, the variation was from 11.9% to 13.2%. It should be emphasized that in each time period, the variation in moisture content was not greater than two percentage points.

Uniform moisture content of the seeds before evaluation of physiological potential is indispensable for standardization of the procedures and for obtaining consistent results. Moisture content should be determined before and after the aging period to check the consistency of the results obtained in the accelerated aging test (Marcos-Filho, 1999). Accentuated differences can negatively affect the intensity of seed deterioration during aging; thus, to ensure uniformity of the test, it is recommended that variations among the samples not be greater than 2.0 percentage points in seed moisture content before and after aging (Marcos-Filho, 1999). This tolerable limit of two percentage points was not exceeded in this study, such that the moisture content did not interfere in the performance of the seed lots during the germination and vigor evaluations.

The results in reference to the seedling emergence test in the field are also shown in Table 1. Comparison among the mean values of emergence percentage resulted in classification of L7 and L8 as having greatest vigor, not differing statistically from L6 and L10. In contrast, lots L2, L5, and L9 were the least vigorous, not differing from L3 and L4. In regard to speed of emergence, lots L7, L8, and L10 exhibited the greatest vigor, not differing from L1 and L6. The least vigorous lots were L5 and L9 (not statistically different from L2). In general, there was no divergence regarding separation of the lots of highest and lowest physiological potential between the tetrazolium test and the accelerated aging tests (TAA and SSAA).

The final destination of the seeds is the field, with the aim of rapid and uniform establishment of stand. Therefore, the results of the vigor tests should be associated with those of seedling emergence in the field, evaluating if different tests allow separation of lots in a way similar to seedling emergence in the field. This efficiency declines as the environmental conditions deviate from those that are most suitable; under very unfavorable environmental conditions, there is no efficient test (Marcos-Filho, 2015a).

Even though there are differences in sensitivity among the tests in identifying differences in seed physiological potential, separation of the seed lots of the cultivar BMX Potência RR was found to be consistent with seedling emergence.

Evaluation of seed physiological potential through resources of image analysis of seedlings (SVIS® and Vigor-S software)

Analysis of variance of the data obtained in the SVIS® and Vigor-S evaluations showed significant values for the effects of lots on all the parameters evaluated. The mean values obtained in computerized analysis of seedling images, SVIS® and Vigor-S, at three days of germination, are shown in Table 2.

The information obtained in SVIS® analyses (vigor index - VI, and seedling length - SL) identified lots L5 and L9 as those of lowest vigor. According to these two parameters, lots L7 (VI) and L8 (VI and SL) were the most vigorous lots, without differing from L10 regarding the vigor index. Studies performed with seeds of soybean (Marcos-Filho et al., 2009; Wendt et al., 2014), peanut (Marchi et al., 2011), common bean (Gomes-Junior et al., 2014), and various other species also showed the sensitivity of SVIS® analyses in separation of seed lots regarding vigor level.

Table 2. Vigor index (VI) and seedling length (SL) obtained in SVIS® and Vigor-S analyses and hypocotyl length (HL) and primary root length (RL) in the Vigor-S system in 10 soybean seed lots, cultivar BMX Potência RR (Cultivar 1). Mean of three experimental periods.

Lot	SVIS®		Vigor-S			
	VI	SL	VI	SL	HL	RL
	index	cm	index	cm		
L1	872 b	9.02 c	660 c	9.97 c	4.14 ab	5.83 d
L2	716 c	6.73 d	528 d	7.53 d	3.28 c	4.25 e
L3	704 c	6.48 d	518 d	7.29 d	3.19 c	4.09 e
L4	700 c	6.47 d	499 d	7.29 d	3.28 c	4.01 e
L5	533 d	4.04 e	349 e	4.59 e	2.26 d	2.33 f
L6	888 b	9.04 c	702 bc	10.15 c	4.02 b	6.12 cd
L7	962 a	10.50 b	820 a	11.73 b	4.35 ab	7.39 b
L8	974 a	11.57 a	880 a	12.85 a	4.49 a	8.36 a
L9	536 d	4.12 e	361 e	4.59 e	2.41 d	2.18 f
L10	927 ab	9.73 bc	736 b	10.87 bc	4.36 ab	6.51 c
CV (%)	3.0	4.2	4.6	4.3	4.0	4.8

Lowercase letters: comparisons between mean values within each column, in separation for each test conducted (Tukey Test, $p \leq 0.05$).

The Ohio State University, USA, proposes a seed vigor classification scale according to the SVIS® software in which a vigor index from 800 to 1000 represents exceptional vigor, from 600 to 799 high vigor, from 400 to 599 good vigor, and from 200 to 399 low vigor (Marcos-Filho et al., 2009). In the present study, the lots of ‘BMX Potência RR’ seeds identified with the highest vigor index achieved values greater than 900, and the lowest vigor index was lower than 599 (minimum of 533).

Furthermore, in Table 2, separation of the lots indicated in the Vigor-S analyses (vigor index, seedling length, hypocotyl length, and root length) was similar to that found for SVIS® (vigor index and seedling length); lots L5 and L9 were identified as those of lowest vigor. The highest vigor lots were L7 and L8, as well as L1 and L10 in the hypocotyl length parameter. High performance of L1 was also observed in the data on seedling emergence speed.

As observed for the SVIS®, it is important that seed production companies define a classification standard based on the indices generated by Vigor-S. This allows adequate interpretation of results obtained in quality control programs, comparable to the interpretation provided when other vigor tests are used. Precise identification of the vigor levels of the samples evaluated constitutes the basis for adoption of suitable management practices of post-harvest seeds.

In general, the SVIS® and Vigor-S automated analyses and the recommended tests (tetrazolium, accelerated aging, and seedling emergence) consistently identified the seed lots of greatest (L7, L8, and L10) and lowest (L5 and L9) vigor. In light of the foregoing, it can be affirmed that the sensitivity of the Vigor-S system for separation (“ranking”) of seed lots of the cultivar BMX Potência RR was comparable to that obtained from use of the SVIS® software and in the tests conducted here for vigor evaluation.

The efficiency of the SVIS® system for separation of the lots was emphasized in various studies and, although use of the Vigor-S system is more recent, its efficiency compared to the tests of conventional use was also confirmed by Castan et al. (2018) in maize seeds and by Medeiros et al. (2019) in common bean seeds.

Vigor-S has additional advantages in relation to the SVIS® in that it provides individualized results of hypocotyl and primary root length and, therefore, detects differences in the degree of development (vigor) of specific parts of the seedlings. In addition, the results in reference to indices of vigor, uniformity, and seedling length provided by Vigor-S can be exported in Excel extensions, allowing better organization and storage of the information obtained and greater ease in later interpretation of the results by seed analysts. At the same time, due to acquisition of images with greater density of spatial resolution (300 dpi) in comparison with SVIS® (100 dpi), there is greater precision of analyses in regard to identification of seedling parts, with less interference of the analyst in correcting imperfections in the marking of seedlings by the software (Castan et al., 2018).

Cultivar 7166 RSF IPRO (Cultivar 2)

Evaluation of seed physiological potential

Analysis of variance of the data indicated significant F values for effects of lots on the parameters evaluated in all the tests conducted. The mean values obtained in the germination and vigor tests and the respective coefficients of variation are shown in Table 3.

The highest percentage of seed germination was observed for lot L20 (97%), not differing significantly from L13 (95%). In contrast, the germination of L11 (79%) and L17 (80%) was statistically inferior to the germination of the other lots evaluated. The germination test is important because it provides information regarding the potential of a sample to germinate under optimal environmental conditions; unfavorable conditions for the seed in the field can hurt seedling emergence.

In regard to vigor evaluated by the tetrazolium test, L20 once more was the best lot, not differing significantly from L13 and L19; the lowest vigor was observed for lot L17.

The results of the traditional accelerated aging test identified the lots of greatest vigor (L12 and L20) and that of lowest vigor (L18). The decrease in the number of normal seedlings in seeds under the traditional accelerated aging test is explained by exposure of seeds to high temperature and relative humidity, which cause intensification of metabolic alterations and inefficiency of enzymes that inactivate the action of free radicals (Tian et al., 2008). In addition, in the accelerated aging test, the seeds undergo anatomical modifications in the hypodermic layer of the testa and collapse of hypodermic cells, changes that are associated with reduction in germination capacity (Silva et al., 2008).

The separation of lots performed by the accelerated aging test with saturated NaCl solution was very similar to that of the tetrazolium test; lots L13 and L20 were indicated as those of greatest vigor, whereas L17 had the lowest

Table 3. Germination (G), tetrazolium-vigor, traditional accelerated aging (TAA 48h) and accelerated aging with saturated NaCl solution (SSAA 72 h), seedling emergence percentage and speed in 10 soybean seed lots, cultivar 7166 RSF IPRO (Cultivar 2). Mean of three experimental periods.

Lot	G	TZ – Vigor	TAA 48 h	SSAA 72 h	Seedling emergence	
	%				%	Index
L 11	79 d	63 e	35 d	70 efg	43 d	2.26 d
L 12	93 bc	78 bc	75 a	81 cd	68 a	3.81 a
L 13	95 ab	87 ab	47 c	88 ab	65 ab	3.50 ab
L 14	89 c	67 de	38 d	68 g	48 cd	2.54 cd
L 15	92 bc	75 cd	51 c	77 def	57 abc	3.04 abc
L 16	92 bc	71 cde	35 d	80 cde	54 bcd	2.83 bcd
L 17	80 d	54 f	32 d	49 h	43 d	2.31 cd
L 18	91 bc	69 de	16 e	69 fg	51 cd	2.62 cd
L 19	92 bc	80 abc	65 b	86 bc	68 a	3.74 a
L 20	97 a	88 a	70 ab	93 a	66 ab	3.67 a
CV(%)	6.6	6.3	9.2	7.0	17.9	8.5

Lowercase letters: comparisons among means within each column, in separation for each test conducted (Tukey Test, $p \leq 0.05$).

vigor. The effect of temperature together with relative humidity in the procedures of the accelerated aging test allowed separation of the lots in different vigor levels. The reduction in seed germination was more accentuated in the TAA than in the SSAA. As emphasized above, this result can be explained by the more stressful conditions seeds are subjected to in the TAA. Classification of the lots based on the results of the SSAA was consistent with separation of the lots through the TAA. Thus, it can be affirmed that the use of saline solution mitigates the severity of the accelerated aging test but does not reduce its efficiency in relation to the traditional procedure. This was also highlighted by Marcos-Filho et al. (2009).

Variation in the moisture content of the seeds of the cultivar 7166 RSF IPRO at the beginning of evaluations was from 7.4% to 10.0%. After the period of the TAA, the variation was from 24.1% to 27.4%, and after the SSAA, the variation was from 11.9% to 13.0%. Within each period, the maximum variation in seed moisture content was two percentage points, that is, within the limits considered suitable for conducting tests.

The data in reference to the percentage and speed of seedling emergence (Table 3) allowed general identification of the lots of greatest (L12, L13, L15, L19, and L20) and of lowest (L11, L14, L17, and L18) vigor. The vigor tests were consistent in separation of lots and sufficiently sensitive to estimate seedling emergence potential.

It should be noted that the percentage of seedling emergence in the field was less than that of germination, confirming the information documented in the literature according to which seedling emergence in the field is more frequently associated with vigor tests. In evaluating soybean seed lots, Wendt et al. (2017) observed that the results of seedling emergence in the field were related to the results of the tetrazolium, accelerated aging, and electrical conductivity tests and analysis of concentration of CO_2 (released in the seed respiration process). Yagushi et al. (2014) likewise found similarity between the data of seedling emergence in the field and the data obtained in the accelerated aging and tetrazolium tests. The environmental conditions during emergence of seedlings in the field in the three time periods of the tests were favorable, with mean temperatures ranging from 20 to 25 °C and relative humidity greater than 60% throughout the period. The irrigation system was activated when necessary.

Evaluation of seed physiological potential through image analysis of seedlings (SVIS® and Vigor-S software)

Analyses of variance of the data obtained through use of the SVIS® and the Vigor-S showed significant values for effects of lots on all the parameters evaluated. The mean values obtained in computerized analysis of seedling images, SVIS® and Vigor-S, at three days of germination are shown in Table 4.

The results obtained through use of the SVIS® (vigor index, seedling length) showed the same sensitivity in separation of lots as Vigor-S (vigor index, seedling length, and hypocotyl length). In the two systems, the lot identified as having greatest vigor was L12, not differing from L20, as well as L19 regarding seedling length and hypocotyl length. The lower vigor of lots L11, L17, and L18 did not differ from L14 in the analyses of the vigor index (SVIS®) and hypocotyl length. The data of primary root length indicated only lots L12 and L20 as those of greatest vigor and L18 as that of lowest vigor.

In general, the results of the automated analyses SVIS® and Vigor-S highlighted the greater vigor of L12, L13, L19, and L20 and the lower vigor of L11, L17, and L18. The inferior performance of lot L14 was also detected in the evaluations of the vigor index (SVIS®), hypocotyl length (Vigor-S), and seedling emergence percentage and speed. The efficiency of the total length of seedlings or of their parts in differentiation of the vigor of seed lots was reported by other authors (Marcos-Filho et al., 2009; Silva and Cicero, 2014).

Studies relating the efficiency of the Vigor-S system compared to tests recommended for evaluation of seed vigor are still few. In contrast, the efficiency of seedling image analysis using the SVIS® compared to traditional methods for determination of vigor has been confirmed in soybean (Marcos-Filho et al., 2009), wheat (Silva et al., 2012), cucumber (Chiquito et al., 2012), sunflower (Rocha et al., 2015), and coffee (Trujillo et al., 2019) seeds, among other species. Though it is a recently developed system, Vigor-S allows accurate and detailed evaluation of seedlings, which is one important argument for its acceptance among seed analysts (Castan et al., 2018; Medeiros et al., 2019).

It should be emphasized that the use of different cultivars did not interfere in the response pattern of seeds to the Vigor-S system. Standardization of a vigor test should preferentially consider the adoption of procedures whose

Table 4. Vigor index (VI) and seedling length (SL) obtained in SVIS® and Vigor-S analyses and hypocotyl length (HL) and primary root length (RL) in the Vigor-S system in 10 soybean seed lots, cultivar 7166 RSF IPRO (Cultivar 2). Mean of three experimental periods.

Lot	SVIS®		Vigor-S			
	VI	SL	VI	SL	HL	RL
	index	cm	index		Cm	
L11	549 de	4.35 de	383 de	4.83 de	2.28 f	2.55 c
L12	823 a	8.12 a	642 a	9.02 a	3.48 a	5.55 a
L13	728 b	6.65 b	552 b	7.48 b	3.11 bc	4.37 b
L14	582 cde	4.71 cd	407 cd	5.29 cd	2.50 ef	2.80 c
L15	611 cd	5.01 cd	446 c	5.63 cd	2.71 de	2.93 c
L16	631 c	5.33 c	452 c	5.99 c	2.98 cd	3.02 c
L17	548 de	4.44 de	348 e	4.93 de	2.30 f	2.63 c
L18	518 e	3.73 e	342 e	4.21 e	2.33 f	1.88 d
L19	751 b	7.10 ab	566 b	7.89 ab	3.24 abc	4.64 b
L20	787 ab	7.52 ab	611 ab	8.48 ab	3.47 ab	5.01 ab
CV (%)	8.7	6.4	5.6	6.4	10.9	7.4

Lowercase letters: comparisons among means within each column, in separation for each test conducted (Tukey Test, $p \leq 0.05$).

results are not significantly affected by genotype (Marcos-Filho, 2015a) and, for that reason, recommended only for comparison among lots of seeds from the same cultivar.

The above results showed that automated seed vigor analysis (Vigor-S) is a rapid and objective option of easy access and low investment cost, constituting a viable alternative for evaluation of seed vigor. Thus, as Marcos-Filho et al. (2009) highlighted for the SVIS®, Vigor-S can also be used as a tool to complement the information provided by other tests, such as accelerated aging and tetrazolium, or even to identify the effects of chemical treatment, physiological conditioning, and situations in which monitoring of germination and of seedling growth at regular intervals is interesting or necessary.

CONCLUSIONS

The results obtained show that automated seedling image analysis through use of the Vigor-S system is efficient for determination of the physiological potential of soybean seeds.

ACKNOWLEDGMENTS

Our thanks to the *Fundação de Amparo à Pesquisa do Estado de São Paulo* (FAPESP) for the scholarship granted (FAPESP process no. 2016/21594-4) and funds for carrying out the study (FAPESP process no. 2006/57900-0). Thanks also to the researchers of the seed and grain technology group of *Embrapa Soja*, especially to Dr. Francisco Carlos Krzyzanowski and Dr. José de Barros França-Neto for granting seeds to carry out this study.

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