Analysis of seedling images to evaluate the physiological potential of soybean seeds

Análise de imagens de plântulas para avaliação do potencial fisiológico de sementes de soja

Análisis de imágenes de plántulas para evaluar el potencial fisiológico de las semillas de soja

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ABSTRACT
The use of artificial intelligence techniques collaborate in laboratory analysis of seeds. In this sense, the objective of this work was to verify the efficiency of the Ground Eye® equipment in evaluating the vigor of soybean seeds and the agronomic performance of soybean varieties 74I77 RSF IPRO and NT 1478SP. For this, experiments were carried out to analyze the germination of soybean seeds. The variables analyzed by the analyst and Ground Eye® equipment were the following: number of normal, intermediate, weak and abnormal seedlings. The tetrazolium, vigor and seedling emergence test under field conditions for soybean cultivars were also performed. For seedling emergence in the field, tetrazolium test and vigor there was no statistical difference between soybean cultivars. The Ground Eye Series L equipment showed a lower number of normal seedlings, number of intermediate seedlings, number of weak seedlings and a higher number of vigorous seedlings when compared to the analyzes carried out manually. Based on the results, the cultivar NT 1478SP showed better performance and may be promising in the field. The Ground Eye L Series equipment is an efficient tool for checking the vigor of soybean seedlings, helping the analyst to perform the tests more quickly.

Keywords: Glycine max L. Groundeye®, vigor.
RESUMO
O uso de técnicas de inteligencia artificial auxiliam nas análises laboratoriais de sementes. Neste sentido, o objetivo do trabalho foi verificar a eficiência do equipamento Ground Eye® na avaliação do vigor de sementes de soja e o desempenho agronômico das variedades de soja 74I77 RSF IPRO e NT 1478SP. Para isso, foram realizados experimentos para analisar a germinação de sementes de soja. As variáveis analisadas pelo analista e equipamento Ground Eye® foram as seguintes: número de plântulas normais, intermediárias, fracas e anormais. O teste de tetrazólio, vigor e emergência de plântulas em condições de campo para as cultivares de soja também foram realizados. Para emergência de plântulas em campo, teste do tetrazólio e vigor não houve diferença estatística entre as cultivares de soja. O equipamento Ground Eye Série L, apresentou menor número de plântulas normais, número de plântulas intermediárias, número de plântulas fracas e maior número de plântulas vigorosas quando comparado com as análises realizadas manualmente. Com base nos resultados, a cultivar NT 1478SP apresentou melhor desempenho, podendo ser promissora no campo. O equipamento Ground Eye Série L é uma ferramenta eficiente para verificar o vigor de plântulas de soja, ajudando o analista a realizar os testes com maior rapidez.

Palavras-chave: Glycine max L. Merril, GroundEye®, vigor.

RESUMEN
El uso de técnicas de inteligencia artificial ayuda en el análisis de semillas en laboratorio. En este sentido, el objetivo del trabajo fue verificar la eficiencia del equipo Ground Eye® en la evaluación del vigor de semillas de soja y el comportamiento agronómico de las variedades de soja 74I77 RSF IPRO y NT 1478SP. Para ello se realizaron experimentos para analizar la germinación de semillas de soja. Las variables analizadas por el analista y el equipo Ground Eye® fueron las siguientes: número de plántulas normales, intermedias, débiles y anormales. También se llevaron a cabo pruebas de tetrazolio, vigor y emergencia de plántulas en condiciones de campo para cultivares de soja. Para la emergencia de plántulas en el campo, prueba de tetrazolio y vigor, no hubo diferencia estadística entre cultivares de soja. El equipo Ground Eye Serie L mostró un menor número de plántulas normales, un menor número de plántulas intermedias, un menor número de plántulas débiles y un mayor número de plántulas vigorosas en comparación con los análisis realizados manualmente. Según los resultados, el cultivar NT 1478SP mostró un mejor rendimiento y puede ser prometedor en el campo. El equipo Ground Eye Serie L es una herramienta eficiente para verificar el vigor de plántulas de soja, ayudando al analista a realizar pruebas con mayor rapidez.

Palabras clave: Glycine max L. Merrill, GroundEye®, fuerza.
1 INTRODUCTION

The soybean crop (Glycine max L.) is considered the most important agricultural commodity in the world. Worldwide production was approximately 371.882 million tons, with a planted area of 121.787 million hectares, and Brazil being the largest producer of the grain with a yield of 3,517 kg ha\(^{-1}\) (Embrapa, 2022).

One of the determining factors for the success of this agricultural production is the use of high-quality seeds. Soybean seed is considered of high quality when it presents high rates of vigor, germination, health, physical and varietal purity, and free of weeds. This quality can be quantified by the vigor test, which is the sum of all seed attributes that favor the rapid and uniform establishment of an initial population in the field (Brazil, 2009).

However, the vigor test presents limitations such as variation in results between laboratories due to the subjectivity of the analysis and the time it takes for results to be ready. The implementation of computational seedling analysis can eliminate the difficulties encountered in the vigor test. Thus, it is necessary to search for technologies to obtain fast and accurate information regarding lot performance in the field and for grain evaluation in storage intended for commercialization (Araújo et al. 2019).

Digital image analysis is a fast and non-destructive method, generating a large amount of data in seed quality evaluation. For image analysis, it is necessary to generate dimensional characteristics such as the area and length of the analyzed object, attributes such as color and texture patterns, and the measurement of counting and frequency of the elements forming the image, pixels. And for image processing, it is necessary to develop computational algorithms that will be systematized for digital image analysis, which will be used for standard comparison (Teixeira 2006).

Studies have been done with X-ray equipment and GroundEye® to evaluate seed quality. Among the computational systems, GroundEye® stands out as equipment that analyzes approximately 300 morphological characteristics of seeds and seedlings. GroundEye® is the most used system for seed analysis. It is composed of an image capture chamber and an analysis software. This chamber is made up of a tray with a transparent acrylic bottom, where the sample will be deposited for analysis, presents a high-
resolution photographic camera, and LED lamps for illumination. The blue coloring on the bottom of the capture chamber is to give greater contrast with the sample of interest. This equipment performs individual image analysis of seeds and seedlings, thus obtaining a wide variety of information related to color, texture, uniformity, vigor, and seedling growth (Andrade et al., 2016).

This technology has been frequently used by researchers. Da Luz et al. (2021), to establish parameters of Passiflora sp. seed lots as a function of fruit maturation stage. In comparing germination with the morphometry of paricarana seedlings, the use of GroundEye® was efficient and accurate in determining vigor (Lima et al., 2018). In research conducted by Xavier et al. 2019 to distinguish predominant characteristics of seeds from different Amaranthus spp. species.

In this sense, the objective of the study was to verify the efficiency of the GroundEye® equipment in evaluating the vigor of soybean seeds of the cultivars 74I77RSF IPRO and NT 1478 SP from seedling images from the germination test, in comparison with analyses performed by an analyst, and to verify the agronomic performance of the soybean varieties 74I77 RSF IPRO and NT 1478SP.

2 MATERIALS AND METHODS

The study was conducted at the seed laboratory of the Federal Institute of Goiás - Campus Iporá. For the experiment, seeds of two soybean cultivars, Bramax Foco 74I77RSFIPRO and NT1478SP, from the 2019/2020 harvest provided by Provigor Análise De Sementes LTDA, Rio Verde, GO were used. Each cultivar was represented by five seed lots stored in a cold chamber at 5°C±2°C and 70% humidity throughout the experimental period. The seeds did not receive any treatments.

The following tests were performed to characterize seed quality: Moisture content: the oven method at 105°C ± 2°C for 24 hours (Brazil, 2009) was used with two replications containing 5g of seeds for each cultivar. The results were expressed as a percentage (wet basis). (correct storage) Germination test: evaluated in four replications of 50 seeds distributed alternately on two germitest papers moistened to 2.5 times their dry weight and covered with an additional moistened paper under the same conditions.
The rolls were then kept in a germinator set at a constant temperature of 25°C with a photoperiod of 16h. The seed germination count was performed five days after sowing, according to criteria established in the Seed Testing Rules (Brazil, 2009). First germination count corresponds to the percentage of normal seedlings on the fourth day after germination test installation. The results were expressed as percentages of normal seedlings for each lot.

Accelerated Aging Test: For this variable, four repetitions of 50 seeds were distributed in a single layer on an aluminum screen fixed in a germination plastic box containing 40 ml of distilled water at the bottom. The boxes containing the seeds were closed and kept at 41°C for 24 hours. Then, the seeds were subjected to the germination and water content test, following the methodology previously described. The evaluation was performed on the fifth day after sowing (Marcos Filho, 2015).

Seedling Growth Test: This test was performed on normal seedlings obtained from the germination and accelerated aging test, five days after sowing. A millimeter ruler was used to take measurements. Manual measurements of the seedlings were taken to determine the length of the longest root, shoot, total length, and the number of branches (Vieira and Carvalho, 1994).

Tetrazolium Test: This test was performed on two subsamples of 50 seeds for each treatment, pre-conditioned on germination paper, type "germitest", moistened and kept for 16 hours in a B.O.D. incubator at 25°C. After this period, the seeds were transferred to plastic cups (50 ml capacity), completely submerged in a 0.075% solution of 2,3,5 triphenyl tetrazolium chloride and kept at 40°C in a B.O.D. growth chamber for three hours. After this period, the seeds were washed in running water and then individually evaluated by longitudinal cutting of the embryonic axis and classified for vigor and viability on a scale of 1 to 8. The vigor and germination potentials were expressed as a percentage (França Neto et al., 1999).

Field Seedling Emergence: This was carried out at the Nova Esperança farm, located in the municipality of Rio Verde - GO. The sowing was performed manually, with five repetitions of 50 seeds per treatment, and the plots were randomly distributed with a spacing of 10 cm between rows. The seedling count was conducted seven days after
installation, and the result was expressed as a percentage of the number of normal seedlings emerged (Brasil, 2009).

Image Analysis: The equipment used was the Ground Eye Series L for germination and accelerated aging tests, with five repetitions of 50 seeds for each cultivar. To capture the images, the seedlings were carefully removed from the "germitest" paper and placed on the equipment's conveyor belt, so that the seedlings did not touch each other. Subsequently, image capture and analysis were initiated. The capture of the germination test images was performed by a high-resolution professional camera contained within the equipment's capture camera (Figure 1A and B). After capturing the seedling images, analysis was performed by the equipment itself (in all cases, the metric unit was centimeter), and seedling germination and vigor were expressed as a percentage.

Figure 1. Analyst organizing the seedlings (A); Separating the seedlings and placing them under the equipment's conveyor belt for image capture.

Source: Authors

Statistical analysis: a completely randomized design with five replicates of 50 seeds was used for the variables. Except for the germination, accelerated aging, and seedling size tests (shoot and root), which were performed in a factorial scheme (2 cultivars x 2 evaluation methods), and field seedling emergence, which was randomized blocks. The results were subjected to analysis of variance and, when significant, means were compared by Tukey's test at a 5% level of probability.

3 RESULTS AND DISCUSSION

The average moisture content of soybean seeds was 12.0 ± 0.4%, which is important for obtaining sample standards. For the germination test, which was performed
with both cultivars and the two evaluation methods, there was interaction for the variable abnormal plants (Table 1), with the Ground Eye Series L equipment detecting a higher percentage of abnormal seedlings in the DO 114320 soybean cultivar compared to the analyst’s evaluation and the NT1478SP soybean cultivar (Table 2).

Table 1. Summary of the analysis of variance for normal seedlings (NS), germination speed index (GSI), intermediate seedlings (IS), weak seedlings (WS), and abnormal seedlings (AS) submitted to the germination test, for two soybean cultivars submitted to two evaluation methods, by an analyst and by Groundeye.

<table>
<thead>
<tr>
<th>Sources of variation</th>
<th>DF</th>
<th>PN</th>
<th>IVG</th>
<th>PI</th>
<th>PF</th>
<th>PA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cultivar (C)</td>
<td>1</td>
<td>1479.2 **</td>
<td>92.5 ns</td>
<td>966 **</td>
<td>5.0 ns</td>
<td>304.2 **</td>
</tr>
<tr>
<td>Evaluation Method (M)</td>
<td>1</td>
<td>627.2 **</td>
<td>4351.3 **</td>
<td>1110 **</td>
<td>3328 **</td>
<td>627.2 **</td>
</tr>
<tr>
<td>C x M</td>
<td>1</td>
<td>115.2 ns</td>
<td>151.3 ns</td>
<td>18.1 ns</td>
<td>7.2 ns</td>
<td>105.8 *</td>
</tr>
<tr>
<td>Repetition</td>
<td>19</td>
<td>70.8 ns</td>
<td>59.7 ns</td>
<td>58.7 ns</td>
<td>36.4 ns</td>
<td>22.6 ns</td>
</tr>
<tr>
<td>Residue</td>
<td>57</td>
<td>38.8</td>
<td>64.5</td>
<td>50.9</td>
<td>23.1</td>
<td>24.9</td>
</tr>
<tr>
<td>Total</td>
<td>79</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

CV (%) = 7.3 19.7 22.6 36.9 44.2

DF = degree of freedom; significant at 0.01. (**) and 0.05 (*) of probability; not significant (ns); CV = coefficient of variation. Source: Authors

Table 2. Percentage of abnormal seedlings for two soybean cultivars submitted to two evaluation methods, by an analyst and by Groundeye.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cultivars</th>
<th>Evaluation method</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of abnormal seedlings</td>
<td>DO114320</td>
<td>Analyst</td>
</tr>
<tr>
<td></td>
<td>9.3</td>
<td>a B*</td>
</tr>
<tr>
<td></td>
<td>NT1478SP</td>
<td>Groundeye</td>
</tr>
<tr>
<td></td>
<td>7.7</td>
<td>a B</td>
</tr>
</tbody>
</table>

* Means followed by the same lowercase letter in the column and uppercase letter in the row do not differ from each other according to the Tukey test (p>0.05). Source: Authors

The percentage of normal seedlings, vigorous seedlings, and intermediate seedlings showed statistical differences separately between the factors. The NT1478SP cultivar showed a higher percentage of normal, vigorous, and intermediate seedlings when compared to the DO 114320 soybean cultivar. However, the cultivars did not differ for the percentage of weak seedlings. Regarding the evaluation method, the Ground Eye
Series L equipment showed a higher percentage of vigorous seedlings, while the analyst verified a higher percentage of normal, intermediate, and weak seedlings (Table 3).

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>PN</th>
<th>PV</th>
<th>PI</th>
<th>PF</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO114320</td>
<td>81,0</td>
<td>39,7</td>
<td>28,05</td>
<td>13,25</td>
</tr>
<tr>
<td>NT1478SP</td>
<td>89,6</td>
<td>41,85</td>
<td>35,0</td>
<td>12,75</td>
</tr>
<tr>
<td>Evaluation method</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Analyst</td>
<td>88,1</td>
<td>33,4</td>
<td>35,25</td>
<td>19,45</td>
</tr>
<tr>
<td>Groundeye</td>
<td>82,5</td>
<td>48,15</td>
<td>27,8</td>
<td>6,55</td>
</tr>
</tbody>
</table>

* Means followed by the same lowercase letter in the column (within the factor) do not differ from each other according to the Tukey test (p>0.05). Source: Authors

In the seedling aging test, there was no interaction between factors, however, there was a statistical difference separately for all variables obtained through this test (Table 4). For the cultivar factor, soybean NT1478SP showed a higher percentage of normal, vigorous, and weak seedlings when compared to soybean DO 114320, which showed 7% more abnormal seedlings compared to soybean NT1478SP. Both soybean cultivars did not differ in terms of abnormal seedlings. The data obtained through manually performed analysis showed a higher percentage of normal and weak seedlings when compared to the equipment, which showed 4% and 12% more vigorous and abnormal seedlings, respectively (Table 5).
Table 4. Summary of variance analysis for normal seedlings (NS), germination speed index (GSI), intermediate seedlings (IS), weak seedlings (WS), and abnormal seedlings (AS) submitted to accelerated aging test, of two soybean cultivars submitted to two evaluation methods, by an analyst and by Groundeye.

<table>
<thead>
<tr>
<th>Sources of variation</th>
<th>DF</th>
<th>PN</th>
<th>IVG</th>
<th>PI</th>
<th>PF</th>
<th>PA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cultivar (C)</td>
<td>1</td>
<td>3001,3 **</td>
<td>352,8 **</td>
<td>353,0 **</td>
<td>288,8 **</td>
<td>845,1 **</td>
</tr>
<tr>
<td>Evaluation method (M)</td>
<td>1</td>
<td>2761,3 **</td>
<td>217,8 **</td>
<td>7,2 ns</td>
<td>4205 **</td>
<td>2832,2 **</td>
</tr>
<tr>
<td>C x M</td>
<td>1</td>
<td>84,1 ns</td>
<td>80,1 ns</td>
<td>28,8 ns</td>
<td>28,8 ns</td>
<td>80,0 ns</td>
</tr>
<tr>
<td>Repetition</td>
<td>19</td>
<td>127,2 ns</td>
<td>89,5 ns</td>
<td>62,9 ns</td>
<td>32,1 ns</td>
<td>76,0 ns</td>
</tr>
<tr>
<td>Residue</td>
<td>57</td>
<td>41,1</td>
<td>50,3</td>
<td>50,6</td>
<td>38,7</td>
<td>34,2</td>
</tr>
<tr>
<td>Total</td>
<td>79</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CV (%)</td>
<td></td>
<td>8,8</td>
<td>30,5</td>
<td>21,9</td>
<td>34,9</td>
<td>29,0</td>
</tr>
</tbody>
</table>

DF = degree of freedom; significant at 0,01 (**) level probability; not significant (ns); CV = coefficient of variation. Source: Authors

Table 5. Percentage of normal seedlings (PN), germination speed index (GSI), vigorous seedlings (VS), intermediate seedlings (IS), weak seedlings (WS), and abnormal seedlings (AS) after accelerated aging test of two soybean cultivars subjected to two evaluation methods, by an analyst and Groundeye.

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>PN</th>
<th>PV</th>
<th>PI</th>
<th>PF</th>
<th>PA</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO114320</td>
<td>67,10 b</td>
<td>21,15 b</td>
<td>30,30 b</td>
<td>15,65 b</td>
<td>23,05 a</td>
</tr>
<tr>
<td>NT1478SP</td>
<td>79,35 a</td>
<td>25,35 a</td>
<td>34,50 b</td>
<td>19,45 a</td>
<td>16,55 b</td>
</tr>
</tbody>
</table>

Means followed by the same lowercase letter in the column (within the factor) do not differ from each other by Tukey's test (p>0.05). Source: Authors

For the tetrazolium and seedling emergence in the field test, there was no statistical difference for the tested cultivars (Table 6). Soybean cultivars DO 114320 and NT1478SP showed similar behavior in terms of germinability and vigor potential through the tetrazolium test and percentage of seedling emergence in the field. These tests were performed only by the analyst (Table 7).

In general, the cultivar NT1478SP showed better performance, probably due to its high protein content, since proteins are basic components for the cell to perform its function, especially in seeds, as they act as reserve substances and catalyze reactions (Schuab
et al., 2006). And this contributes to a greater seed vigor, that is, the emergence will be faster and more uniform, resulting in the development of normal seedlings with a wide range of field conditions (Marcos-Filho, 2005). Carvalho et al. (2021), aiming to evaluate the adaptability of conventional soybean genotypes in the southwest region of the State of Goiás (municipalities of Rio Verde, Montividiu, and Santa Helena de Goiás, in the 2017/2018 harvests, found that the NT1478SP cultivar had higher protein content and was responsive to grain yield. However, lots of soybean seeds with high levels of total soluble protein may also indicate seeds with advanced aging and deterioration levels (Santos, 2022).

Table 6. Summary of the analysis of variance for field seedling emergence (FSE), tetrazolium test - germinability potential (TZ G), and vigor (TZ G) for two soybean cultivars.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>DF</th>
<th>EPC</th>
<th>TZ G</th>
<th>TZ vigor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cultivar</td>
<td>1</td>
<td>2.4</td>
<td>390.6</td>
<td>6.4</td>
</tr>
<tr>
<td>Repetition</td>
<td>19</td>
<td>28.1</td>
<td>262.9</td>
<td>35.3</td>
</tr>
<tr>
<td>Residue</td>
<td>19</td>
<td>25.0</td>
<td>284.3</td>
<td>28.3</td>
</tr>
<tr>
<td>Total</td>
<td>39</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

CV (%) = 5.6, 18.7, 5.9

DF = degree of freedom; not significant (ns); CV = coefficient of variation

Source: Authors

Table 7. Percentage of field seedling emergence (FSE), percentage of germinability potential through tetrazolium test (TZ G), and vigor (TZ vigor) in two soybean cultivars.

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>EPC</th>
<th>TZ G</th>
<th>TZ vigor</th>
</tr>
</thead>
<tbody>
<tr>
<td>74I77 RSF IPRO</td>
<td>86.8</td>
<td>A*</td>
<td>90.4</td>
</tr>
<tr>
<td>NT1478SP</td>
<td>90.0</td>
<td>A</td>
<td>89.6</td>
</tr>
</tbody>
</table>

*Means followed by the same lowercase letter in the column do not differ from each other according to the Tukey test (p>0.05). Source: Authors

Regarding the data, a higher percentage of vigorous seedlings, a lower percentage of weak and abnormal seedlings obtained by the Ground Eye Series L system compared to the data observed by the analyst in the germination and accelerated aging tests may be related to the uniformity pattern adjusted in the equipment. For a reliable digital analysis
of features represented by an image, techniques that treat and eliminate false aspects that may be erroneously detected and interpreted should be used. Among them, the fine adjustment variable is employed to reduce unwanted pixels; that is, it is a process intended to reduce the shape to a more simplified version (skeleton) while maintaining the essential characteristics of the original object, even considering small imperfections (Russo et al., 2017). It is a variable that indicates how many adjustments were necessary to prepare the image of the object for a perfect analysis.

In studies developed by Acha and Vieira (2020), it is demonstrated that the fine adjustment is positively correlated with contour deformation ($r = 0.87$). This result was observed when correlating data from the thinning variable with contour irregularity and contour deformation in all tested treatments. However, the authors verified that the analysis in the GroundEye® indicates whether the coating (sand + silica) was able to alter the seed's initial shape, as well as the possibility of classification and standardization of coated seeds, reducing the chances of errors made by the evaluator.

Xavier et al. (2019), aiming to evaluate and distinguish the morphological, chemical, and physiological characteristics of Amaranthus sp. seeds, state that through GroundEye® and NIR, it is possible to distinguish the three species based on geometric characteristics, predominant coloration, and chemical components. According to Guedes et al. (2011), the area, perimeter, maximum and minimum diameters, circularity, and roundness of soybean seeds can be accurately determined using digital analysis, compared to manual evaluation methods. In this sense, we must understand the purpose of software, in this case, we want technology to support the analyst's work, obtaining quick responses, saving time to perform the task, and obtaining standardized samples (Prado Junior et al., 2020).

Our results reveal the potential for analyzing soybean seedling vigor based on software, but for better assessment of robustness, even considering the quality of the coupled sensors, further studies and approaches are required for a better understanding of the system.
4 CONCLUSION

● The soybean seed NT 1478 SP showed higher agronomic performance.
● The Ground Eye Series L equipment was efficient in determining the vigor of soybean seeds.
● Manual measurements also assist us in determining the quality parameters of soybean seeds.

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