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NOTE

Validation of the Vigor-S image analysis system for the characterization of phytotoxicities in soybean seedlings

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ABSTRACT: Phytotoxic effects induced in soybean seedlings by exposure to various pesticides can lead to shortened and thickened hypocotyls, taproot atrophy, and reduced development or absence of secondary roots. This study aimed to validate the Vigor-S image analysis system for characterizing phytotoxic effects on soybean seedlings resulting from seed treatment using different products. Soybean seeds of cultivar BRS 284 received three seed treatments: cyproconazole fungicide, which causes hypocotyl shortening; glyphosate herbicide, which results in reduced root system; and control (without treatment). The experiment used a randomized complete block design with six replications. Seed physiological quality was assessed by the germination test, seedling length by the traditional method and also determined by the Vigor-S system method. There was a significant positive correlation when comparing seedling length parameters between the conventional and Vigor-S methods. It was concluded that seedlings exhibiting phytotoxicity symptoms from exposure to glyphosate herbicide and cyproconazole fungicide had reduced seedling length. The Vigor-S system effectively detects both types of phytotoxicity in soybean seedlings, which could have implications for agricultural practices.

Index terms: computerized image analysis, *Glycine max* (L.) Merrill, seedling length.

RESUMO: Efeitos fitotóxicos induzidos em plântulas de soja devido à exposição a diferentes pesticidas podem resultar em encurtamento e engrossamento de hipocótilo, atrofia na raiz principal e redução do desenvolvimento ou ausência de raízes secundárias. Este trabalho buscou validar o sistema de análise de imagens Vigor-S para caracterizar os efeitos fitotóxicos em plântulas de soja, provenientes do tratamento de sementes com diferentes produtos. Sementes de soja da cultivar BRS 284 foram submetidas a três tratamentos: fungicida ciproconazol, que resulta em encurtamento do hipocótilo; herbicida glifosato, que resulta em redução no desenvolvimento do sistema radicular; e controle (sem tratamento). O delineamento experimental consistiu em blocos ao acaso com seis repetições. A qualidade fisiológica das sementes foi avaliada por meio de testes de germinação, e o comprimento das plântulas foi medido tanto pelo método tradicional quanto pelo sistema de análise de imagens Vigor-S. Observou-se uma correlação positiva significativa ao comparar os parâmetros de comprimento das plântulas entre os métodos tradicional e Vigor-S. Concluiu-se que plântulas que apresentam sintomas de fitotoxicidade causados pela exposição ao herbicida glifosato e ao fungicida ciproconazol apresentam comprimento de plântulas reduzido. O sistema Vigor-S efetivamente detecta ambos os tipos de fitotoxicidade em plântulas de soja.

Termos para indexação: análise computadorizada de imagens, *Glycine max* (L.) Merrill., comprimento de plântulas.

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INTRODUCTION

Soybean crop plays a role of great economic importance for Brazilian agribusiness, meeting both domestic demands and exports. Due to its importance in Brazil and in the world, there are searches for constant improvements in seed quality, as a seed with high vigor expresses the high production potential of the crop (Krzyzanowski et al., 2018).

Using high-quality seeds is the basis for the establishment, uniformity, and agronomic performance of the crop. A high-quality seed must have attributes in terms of physiological (germination and vigor), sanitary (free of pathogens and pests), physical (free of impurities) and genetic (without varietal mixtures) qualities (França-Neto et al., 2016).

Some practices routinely employed in the seed production system can be sources of phytotoxicity problems to soybean seedlings, which may compromise seed performance. Practices such as seed treatment with fungicides and insecticides, as well as pre-harvest desiccation with some specific herbicides, can result in phytotoxicity (Daltro et al., 2010; Toledo et al., 2014; Abati et al., 2020). In addition, industrial seed treatment, when poorly conducted and depending on the quality level of the lots, can cause phytotoxicity problems to seedlings (Brzezinski et al., 2017).

According to França-Neto et al. (2000), phytotoxic effects interfere with seed physiology, reducing the percentage of germination and seedling emergence, and may cause thickening, shortening, longitudinal fissures and hypocotyl stiffness, root system atrophy, curvature of the taproot, absence or poor development of secondary roots, retardation in the development and growth of the aerial part, factors that can reduce the stand establishment and crop yield.

A possible source of phytotoxicity in the operation of soybean seed treatment refers to the use of products at doses above the recommended one. In addition, some fungicides are more phytotoxic than others. Among the fungicides, triazoles are considered to be phytotoxic to soybean seedlings when used in seed treatment. A striking example of this was observed with the contamination of the fungicide Rhodiauram (thiram) by bromuconazole, which also resulted in root shortening, but the main symptoms were detected by severe shortening and thickening of the hypocotyls (França-Neto et al., 2000).

Among the herbicides used in pre-harvest, glyphosate is considered an extremely phytotoxic agent, which can result in serious problems of abnormality of soybean seedlings, through the severe shortening of their root system and abortion of secondary roots (Daltro et al., 2010; Toledo et al., 2012; Toledo et al., 2013; Toledo et al., 2014).

One of the tests that can be used to detect possible symptoms of phytotoxicity in seedlings is the seedling length test (Krzyzanowski et al., 2020a). The test shows values in centimeters of hypocotyl and roots with possible shortening and atrophy, allowing the comparison of seedlings with phytotoxicity and normal seedlings (França-Neto et al., 2000).

Several tests have been commonly used to determine vigor in seeds, such as the tetrazolium test (França-Neto and Krzyzanowski, 2022), accelerated aging, electrical conductivity test (Krzyzanowski et al., 2020b), among others. Some of these tests are being conducted using the facilities and precision promoted by computerized image analysis.

Recently, a new software for the automated evaluation of soybean seed vigor, called Vigor-S, has been reported. This software allows the determination of the vigor index and the length of soybean seedlings with great speed and precision. In addition, systems that use computerized image analysis have the advantage of eliminating possible errors made by humans, are reliable, and make it possible to save the image for later comparison with other images (Gomes-Junior, 2020).

Vigor-S efficiency in the evaluation of vigor has been confirmed for seeds of maize (Castan et al., 2018), common bean (Medeiros et al., 2019) and soybean (Rodrigues et al., 2020). In soybean seeds, the results revealed consistency of the Vigor-S analysis in the identification of seed lots of higher and lower vigor, in comparison with the results of the seedling emergence test in the field and other traditionally used vigor tests, such as the accelerated aging test and the tetrazolium test (Rodrigues et al., 2020).

As mentioned earlier, seedling length testing can be used to assess these problems. As for Vigor-S, the question remains: in addition to determining seed vigor, can the test be used to diagnose possible phytotoxic effects arising from the treatment of soybean seeds and the application of foliar desiccants in pre-harvest? Oliveira et al. (2021) partially answered this question when they found that Vigor-S showed good sensitivity to detect the possible phytotoxic effects of soybean seed treatment with fungicides (carbendazim and thiram), insecticides (imidacloprid and thiodicarb) and micronutrients (cobalt and molybdenum) and their combinations.

Taking these aspects into consideration, the objective of the present study was to validate the use of the Vigor-S system, aiming at the determination of possible phytotoxic effects on soybean seedlings, resulting from the exposure of seeds to the treatment with fungicide (cyproconazole) and also to the herbicide glyphosate, simulating conditions that may occur in pre-harvest.

MATERIAL AND METHODS

Soybean seeds of the BRS 284 cultivar, with germination higher than 90%, were used for the study. The seeds were subjected to two active ingredients to simulate symptoms of phytotoxicity in seedlings: the fungicide cyproconazole and the herbicide glyphosate. In addition to these treatments, the control treatment (control) consisted of untreated seeds.

The experimental design consisted of three treatments applied to the seeds (control, cyproconazole and glyphosate). These treatments were distributed in a randomized complete block design, with six replications per treatment, totaling 18 experimental units. In the treatment with the fungicide cyproconazole, from the triazole group, the dose used was 100 g of the product for every 100 kg seeds. For the treatment with the herbicide glyphosate, the seeds were initially placed on rolls of germination paper moistened with a solution of the herbicide (glyphosate 720 $g.kg^{-1}$), at a concentration of 0.6% in water of the acid equivalent (AE). The volume applied corresponded to 2.5 times the mass of the dry substrate (germination paper). Each roll contained fifty soybean seeds, which were placed in a germinator and kept under conditions of absence of light for a period of 16 hours for soaking. After this period, the seeds previously soaked in the glyphosate solution were transferred to other germination rolls, following the methodology detailed below.

Due to the initial moisture content of 9.0% in the seeds (determined using the Agrosystem-GAC® 2100 digital moisture meter), in order to prevent damage resulting from rapid imbibition, the seeds were pre-conditioned before the glyphosate pre-imbibition tests (as reported above) and the subsequent germination and seedling length tests, according to the prescriptions contained in Additional Instruction No. 70 of the Rules for Seed Testing (RST) (Brasil, 2009). The seeds were placed on aluminum screens and put into plastic boxes containing 40 mL of distilled water. These boxes were kept in a germinator for a period of 24 hours, at a constant temperature of 25 °C. After this step, the moisture content of the seeds increased to about 20%. Subsequently, the seeds were subjected to quality evaluation tests, as detailed below.

Germination test: conducted with four subsamples of 50 seeds for each replication per treatment. The seeds were placed on rolls of germination paper, at 25 °C, moistened with a volume of water equivalent to 2.5 times the weight of the dry substrate. Germination was evaluated in two readings. The first at five days after sowing, and the second at the eighth day. These readings followed the procedures recommended by RST (Brasil, 2009), and germination percentage was recorded according to the guidelines.

Seedling length test: conducted with five subsamples, each containing twenty seeds per paper roll per treatment. The seeds were distributed in two alternated rows with ten seeds in each row, with the micropyle directed to the lower part of the paper. The germination paper rolls were moistened with 2.5 times the weight of the dry substrate, consisting of two sheets at the base and one covering the seeds. Subsequently, the rolls were prepared, grouping the five units with rubber ties, and wrapped in plastic bags to maintain moisture. These rolls were then placed upright inside the germinator, kept under conditions of absence of light at 25 °C for three days to produce the seedlings. The resulting seedlings were scanned for further analysis using the Vigor-S computer program. This same methodology was repeated in other germination rolls, which were left to germinate in the dark for five days at 25 °C (traditional procedure). This allowed the seedling length test to be carried out according to the methodology described by Krzyzanowski et al. (2020a).

Evaluation by Vigor-S: the seedlings were obtained according to the previously described methodology, after a germination period of three days in the dark, at a temperature of 25 °C. Then, the seedlings were transferred from the germination paper rolls to a dark blue sheet of paper with dimensions of 30 cm x 22 cm, corresponding to the size of the usable area reached by the inverted scanner. This sheet was placed on the inner platform of the metal box. For the evaluation, a group of 20 seedlings was scanned in an image obtained. All scanned images were stored in a specific folder on the computer's hard drive, allowing for effective organization and easy access for subsequent analyses.

The images captured by the scanner were processed using the Vigor-S software, installed on a DELL® computer, with a core i5 processor, 2.30 GHz CPU and Windows 7® operating system. With this software, the images were analyzed in an automated and accurate manner. After processing, the software performed the following steps: first, the hypocotyl of the plants was highlighted in blue, while the primary root was identified in red. Abnormal seedlings were detected and recorded by the system, as well as seeds that did not germinate. After completion of this process, the software generated values for vigor index, growth and uniformity of seedling development, ranging from 0 to 1000. In addition, the software provided measurements of hypocotyl size, root size, and total seedling length, expressed in centimeters (Figure 1).

After the analysis was completed by the system, the resulting values were automatically transferred to an Excel spreadsheet. The time elapsed between the digitization of the seedlings and the completion of the analysis in the software was less than 5 minutes. Regarding the data obtained by the Vigor-S software, this study focused exclusively on the values of seedling length and its components, in particular, hypocotyl length and root length.

Statistical analyses: Data related to germination and seedling length (SL), hypocotyl length (HL) and root length (RL), obtained using both the traditional measurement method and the Vigor-S software, were subjected to analysis of variance according to the statistical model of the experimental design. Means were grouped using Tukey's test, with a significance level of 5%. The validation of the efficacy of the Vigor-S software to determine the attributes SL, HL and RL was conducted by means of regression analyses, establishing a relationship between the attributes obtained by the traditional method for measuring seedling length and those obtained by the software.

Figure 1. Analyzed images of soybean seedlings of the cultivar BRS 284, with specific demarcation of the root-hypocotyl axis, illustrating the data obtained by the Vigor-S system: vigor index, seedling uniformity and values of seedling length.

RESULTS AND DISCUSSION

According to the determination of seedling length, it was possible to observe that the control treatment had the highest means of seedling length (SL), hypocotyl length (HL) and root length (RL) (Table 1). Exposure of seeds to pesticide residues, including triazole fungicides (such as cyproconazole) or herbicides (such as glyphosate), can have a negative impact on the values of seedling length and its components (hypocotyl length and root length). It is worth noting that triazole fungicides can cause more pronounced phytotoxicity symptoms in hypocotyls, resulting in thickening and shortening of these structures (França-Neto et al., 2000). Similarly, herbicides such as glyphosate can cause more pronounced phytotoxic damage to seedling roots, leading to taproot atrophy and absence or severe shortening of secondary roots (Daltro et al., 2010).

The results obtained by the Vigor-S software (Table 2), as well as the results obtained by the germination test (Table 3), showed patterns similar to those obtained by the traditional method. In other words, the treatments with the application of the fungicide cyproconazole and the herbicide glyphosate showed lower germination and SL values compared to the control group (untreated seeds), resulting in similar effects for the values of HL, with shortened and thickened hypocotyls, and RL, as the root was also shortened and there was absence of secondary roots. The results confirm the theory of Zablotowicz and Reddy (2004), who state that when there is the presence of molecules of some pesticides, they can concentrate in the meristematic zones of the seedlings, inhibiting the formation of essential amino acids, which are involved in the synthesis of proteins, responsible for the seedling growth process. Thus, once the meristematic zones are compromised, the physiology of the seedling is affected, leading to low germination rate and slow seedling growth.

Table 1. Seedling length (SL), hypocotyl length (HL) and root length (RL), determined by the traditional method of determination of seedling length, performed on soybean seedlings of the BRS 284 cultivar, five days after setting up the tests, after being subjected to treatments with the fungicide cyproconazole and the herbicide glyphosate.

Treatment	Means of measurements by the traditional method (cm)		
	SL	HL	RL
Cyproconazole	9.66 b[1]	1.78 c	7.87 b
Glyphosate	4.82 c	3.22 b	1.61 c
Control	28.37 a	8.74 a	19.62 a
CV (%)	8.62	11.20	8.47

[1]Values followed by the same letter in the column belong to the same group (Tukey test, $p \leq 0.05$).

Table 2. Seedling length (SL), hypocotyl length (HL) and root length (RL), determined by the Vigor-S software, performed on soybean seedlings of the BRS 284 cultivar, three days after setting up the test, after being subjected to treatments with the fungicide cyproconazole and the herbicide glyphosate.

Treatment	Means of estimates by Vigor-S software (cm)		
	SL	HL	RL
Cyproconazole	3.62 b[1]	1.39 c	2.21 b
Glyphosate	3.16 b	2.08 b	1.14 c
Control	7.58 a	2.45 a	5.12 a
CV (%)	8.94	11.13	10.00

[1]Values followed by the same letter in the column belong to the same group (Tukey test, $p \leq 0.05$).

Table 3. Results of the germination test, showing the percentages of normal and abnormal seedlings, carried out on soybean seeds of the BRS 284 cultivar, after being subjected to treatments with the fungicide cyproconazole and the herbicide glyphosate.

Treatment	Germination (%)	
	Abnormal seedlings	Normal seedlings
Cyproconazole	86.1 a[1]	13.9 b
Glyphosate	87.3 a	12.8 b
Control	4.9 b	95.1 a
CV (%)	9.29	13.60

[1]Values followed by the same letter in the column belong to the same group (Tukey test, p ≤ 0.05).

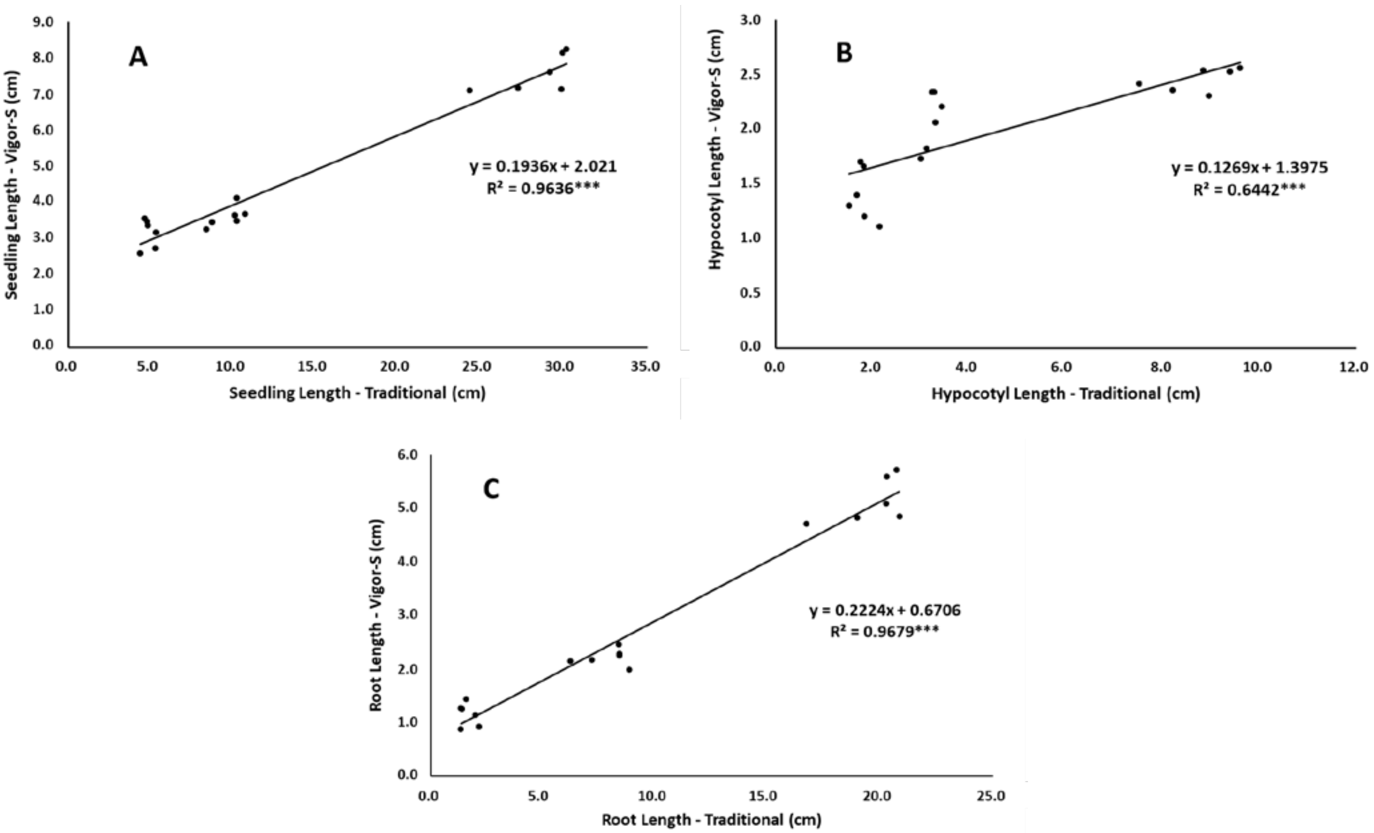

Figure 2. Regression analyses between seedling length (A), hypocotyl length (B) and root length (C) obtained by the traditional method of determination of seedling length and its corresponding estimates by the Vigor-S software. Seedlings obtained from seeds of the soybean cultivar BRS 284, after exposure to three treatments evaluated in this article.

When comparing the regression analyses between the measurements of seedling length and its components, performed by the traditional method and by Vigor-S, it was found that the coefficients of determination obtained were remarkably high and highly significant: 0.9636 for seedling length (Figure 2A); 0.6442 for hypocotyl length (Figure 2B); and 0.9679 for root length (Figure 2C). These results highlight the feasibility and reliability of the Vigor-S method, indicating that it can be successfully used to identify possible phytotoxic effects resulting from exposure of soybean seedlings to triazole fungicides (such as cyproconazole) and herbicides (such as glyphosate). In addition to reducing human error, the Vigor-S method also decreases the time to obtain results. These findings reinforce the conclusions presented by Rodrigues et al. (2020) and Oliveira et al. (2021), who highlighted the efficacy and sensitivity of Vigor-S as a valuable tool to evaluate potential phytotoxic effects resulting from the treatment of soybean seeds with various active ingredients.

CONCLUSIONS

Vigor-S software is effective in estimating the parameters used to diagnose phytotoxicity in soybean seedlings grown from seeds treated with the fungicide cyproconazole or exposed to residues of the herbicide glyphosate. In addition, the treatment of soybean seeds with the fungicide cyproconazole results in a reduction in seedling length, especially with regard to the hypocotyl, and exposure of soybean seedlings to the herbicide glyphosate results in a reduction in seedling length, especially with regard to the root system.

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