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Validation of the methodology of the germination test using a rolled paper plus vermiculite for treated soybean seeds

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ABSTRACT: The use of an appropriate germination method is crucial to allow expression of the germination potential of the seed lot, especially when the seeds are treated with phytosanitary products, since phytotoxicity can occur in some substrates and under some conditions and the germination values will be underestimated. In this context, the aim of this study was to evaluate the methodology of germination in a rolled paper plus vermiculite (RP+V) and the possibility of its statistical validation for routine use in laboratories. Four seed lots of two cultivars with different vigor levels and chemical insecticide treatments (control, thiamethoxam + cyantraniliprole, clothianidin + fipronil, and imidacloprid + thiodicarb, all seeds were treated with fungicide) were used. The samples were prepared and sent to 13 laboratories accredited by the *Ministério da Agricultura e Pecuária* (MAPA) for germination assessment with RP+V. The collected data were analyzed using statistical methodologies to assess the repeatability and reproducibility of the method, as well as its precision and accuracy. The data from normal soybean seedlings evaluated using the rolled paper plus vermiculite (RP+V) methodology show repeatability, reproducibility, precision, and accuracy within the critical parameters of 1% and 5%. The proposed methodology is reliable and can be routinely implemented in evaluation of soybean seeds, especially those treated with phytosanitary products.

Index Terms: chemical seed treatment, quality control, reproducibility, repeatability, substrate.

RESUMO: O uso de uma metodologia de germinação adequada é crucial para que possibilite a expressão do potencial germinativo do lote de sementes, principalmente quando estão tratadas com produtos fitossanitários, pois em alguns substratos e condições podem ocorrer fitotoxidez e os valores serem subestimados. Neste contexto, objetivou-se neste estudo avaliar a metodologia de germinação em rolo de papel mais vermiculita (RP+V) e a possibilidade de validação estatística dessa para uso em laboratórios de rotina. Foram utilizados quatro lotes de sementes de duas cultivares, com diferentes níveis de vigor e tratamentos químicos inseticidas (controle, tiametoxam + ciantraniliprole, clotianidina + fipronil e imidacloprido + tiodicarbe, todas sementes foram tratadas com fungicida). As amostras foram preparadas e enviadas para 13 laboratórios credenciados no Ministério da Agricultura e Pecuária (MAPA), Brasil, para análise de germinação por RP+V. Nas análises dos dados coletados foram utilizadas metodologias estatísticas para avaliar a repetibilidade e a reprodutibilidade do método, bem como avaliar sua precisão e acurácia. Os dados de plântulas normais de soja avaliadas com a metodologia de rolo de papel com vermiculita (RP+V) apresentam repetibilidade, reprodutibilidade, precisão e acurácia dentro dos parâmetros críticos de 1% e 5%. A metodologia proposta é confiável para ser implementada de forma rotineira na avaliação de sementes de soja, sobretudo para as tratadas com produtos fitossanitários.

Termos para indexação: tratamento químico de sementes, controle de qualidade, reprodutibilidade, repetibilidade, substrato.

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INTRODUCTION

In Brazil, germination tests for the purpose of marketing seed lots are conducted according to the Rules for Seed Testing (*Regras para Análise de Sementes* (RAS)), defined by the *Ministério da Agricultura e Pecuária* – (MAPA) (Brasil, 2009), so as to obtain comparable results among laboratories and minimize possible discrepancies among results from different laboratories.

The methodologies of germination in a rolled paper (RP) and in between sand (BS) (Brasil, 2009) are widely used in assessment of soybean seeds, as they are official methodologies for issuing report of analysis that allow seeds to be traded if the minimum values stipulated in Brazilian legislation are achieved (Brasil, 2013).

However, there are reports from laboratories performing routine seed analysis regarding underestimation of seed lot quality as a result of the substrate used, especially when seeds are treated with phytosanitary products, due to possible phytotoxicity. That often compromises the relationship between laboratory results and true results observed, above all under field conditions. Among the diverse factors that affect the results of germination tests are the active ingredients (a.i.) used in chemical treatment of the seeds, and the substrate in the germination test (Carvalho et al., 2020, Rocha et al., 2020; Tunes et al., 2020; Carvalho et al., 2021; Rocha et al., 2023).

Currently, for technical and logistical reasons, chemical seed treatments are often conducted before storage, especially through the industrial seed treatment (IST) modality (Medeiros et al., 2023; Reis et al., 2023). Despite the advantages of chemical seed treatments, storage of treated seeds for extended periods can harm seed physiological quality depending on the combinations of factors involved, including the phytosanitary product used and the final composition of the slurry, e.g., the use of neonicotinoid insecticides in stored soybean and maize seeds (Santos et al., 2018; Oliveira et al., 2020; Moraes et al., 2022; Carvalho et al., 2022; Santos et al., 2023). In addition to these factors, the methodology and the substrates used in the tests also affect evaluation of seed lots treated with chemical molecules (Rocha et al., 2020). In this respect, adjustments in the methodology and execution of the germination test are necessary to increase its accuracy and representativeness, especially in the context of seeds treated with chemical molecules.

The substrate used in the germination test affects the results, due to its structure, aeration, water holding capacity, and susceptibility to infestation by pathogens, among other factors. Therefore, the choice of the substrate should correspond to the physiological demands of germination of each species, taking seed size and shape into consideration (Brasil, 2009).

To evaluate germination of soybean seeds, the substrates officially recommended are RP and BS (Brasil, 2009). However, Tunes et al. (2020) observed negative effects from products containing imidacloprid on soybean seeds, mainly in the RP substrate. For Rocha et al. (2020), treatments with insecticide molecules affected germination and seedling evaluation, with greater intensity in relation to fungicides, especially in the RP germination test. Thus, the use of vermiculite may be advantageous and relevant in certain contexts, especially for seeds treated with phytosanitary products (Tunes et al., 2020; Rocha et al., 2023).

Vermiculite is mineral similar to mica, mainly composed of hydrated aluminum and magnesium silicates. Its surface properties, including surface area, hydrophobicity, porosity, and negative surface charge, make it an appropriate absorbent and carrier material (Nunes et al., 2023). Consequently, there is the possibility of vermiculite adsorbing chemical compounds that may be excessively concentrated when only in the RP, which will mitigate possible abnormalities in the seedlings, brought about by phytotoxicity (Rocha et al., 2020; Tunes et al., 2020).

Given the wide adoption of soybean seeds treated with phytosanitary products, studies are necessary to validate new methodologies, such as the use of vermiculite in germination, to lessen problems with phytotoxicity in evaluations in this scenario. That will contribute to satisfactory correlation between the results of germination tests conducted in laboratories and the true quality observed in grain production fields, together with economic and operational viability in laboratories.

The purpose of validating methodologies is to establish a system that allows the adoption of new testing methods, or comparison of similar tests, or review of existing seed quality evaluation methods. Such methods should be applied by various laboratories, ensuring repeatability and reproducibility, as well as the precision and accuracy of the testing method (ISTA, 2008; Rocha et al., 2023). Generally in Brazil, the methodology used to validate seed analysis testing is the Method Validation for Seed Testing of the International Seed Testing Association (ISTA) (ISTA, 2006). That way, with validation of the methodology, it can be used in a reliable way on a large scale in routine testing laboratories.

Therefore, the aim of this study was to evaluate the use of the methodology of germination in a rolled paper with the addition of vermiculite (RP+V) and check the possibility of statistical validation of the methodology for use in routine testing laboratories for soybean seeds.

MATERIAL AND METHODS

The present study was conducted in the central seed research laboratory of the *Departamento de Agricultura*, *Escola de Ciências Agrárias de Lavras* (ESAL) of the *Universidade Federal de Lavras* (UFLA), Lavras, MG, Brazil, and in partner seed testing laboratories.

The activities of the present study were carried out as recommended in the Method Validation for Seed Testing (ISTA, 2006) and were organized and summarized in an organizational chart (Figure 1), divided into three steps.

Step 1 – Choice and initial characterization of the quality of the soybean seed lots and homogeneity test

In the first step, called initial procedures, seeds from two soybean cultivars were used, one with a greater tendency and the other with a lesser tendency to sensitivity to seed treatment with insecticides and storage, according to the

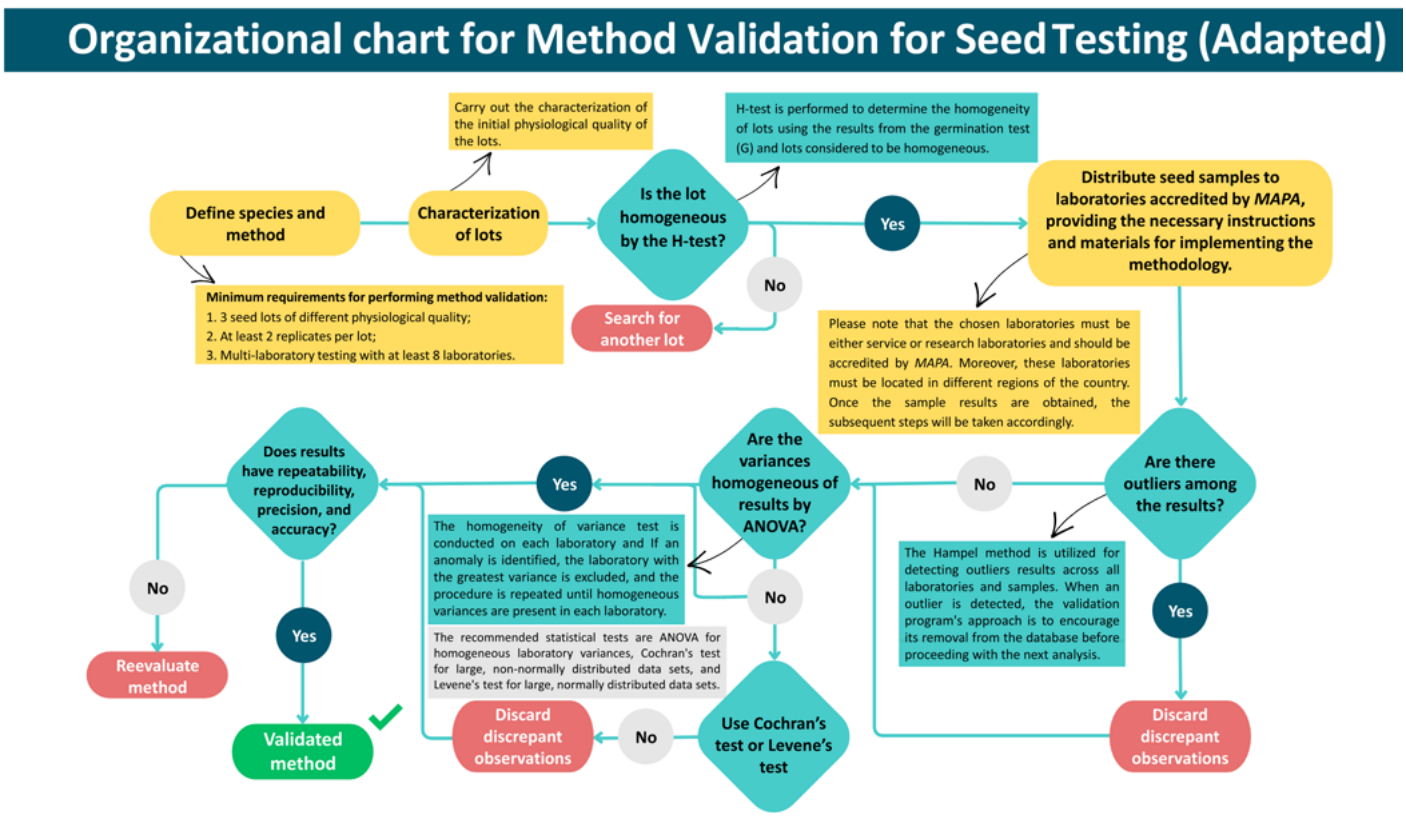

Figure 1. Organizational chart of the summarized procedures for validation of methodologies for seed analysis, adapted from the ISTA (2006).

breeding companies and commercial data. Two seed lots with different levels of physiological quality were selected for each cultivar (Table 1), which were initially evaluated and checked for homogeneity.

The following physiological tests were carried out for characterization and confirmation of physiological quality:

Germination in a rolled paper (RP): eight replications of 50 seeds per treatment were placed above of one damped paper towel, covered with a second damped, and rolled into a cylinder. The amount of water in the towels was 2.5 g of water per g of towel. The rolls were kept in a Mangelsdorf type germinator regulated to the temperature of 25 °C ± 2 °C. Evaluations were made at five (FC) and eight (G) days after sowing, considering the percentage of normal seedlings(Brasil, 2009; ISTA, 2024).

Accelerated aging (AA): Plastic boxes fitted with suspended aluminum screens were used. A layer of seeds was added over the entire screen, followed by the addition of water of 40 mL. Subsequently, they were placed in a chamber at 41 °C ± 0.5 °C for 48 hours (Marcos-Filho, 2020). After this period, sowing was conducted following the procedure outlined in the germination test (Brasil, 2009; ISTA, 2024), with the assessment of normal seedlings conducted 5 days after sowing, and the outcomes was presented as percentages.

Emergence in substrate with controlled conditions (ES): seeds were sown in a substrate composed of sand and clayey soil in a 2:1 ratio, respectively, with four replications of 50 seeds, and the substrate was irrigated to 60% of its water-holding capacity. After sowing, the plastic trays (51 × 30 × 9 cm) containing the substrate were placed in a plant growth chamber at a temperature of 25 °C ± 2 °C, in an alternating (12 h) light and dark regime for 10 days, with final count of emerged seedlings expressed as percentage (Krzyzanowski et al., 2020).

The H Test was carried out with the results of the RP test at eight days to check the homogeneity of the seed lots (Brasil, 2009). Only the lots considered homogeneous were selected for the second step.

Step 2 – Preparation and distribution of the samples and laboratory procedures

In the second step, the seeds were treated with phytosanitary products, the samples were distributed, and instructions for the procedures to be carried out and the materials selected for conducting the test were sent to the seed testing laboratories accredited by *MAPA*.

The seed treatment process was carried out using the Momesso Arktos Laboratório L2K BM device, with calibration setting of 20 hertz in the equipment inverter and application followed by homogenization for 20 seconds to simulate industrial treatment in batches. All the treatments received a standard slurry that included the fungicide Maxim Advanced® and polymer *Biocroma vermelho Biogrow®* (Table 2), as well as different insecticides (Table 3).

The combinations of the four lots with the four seed treatments were identified with sequential protocol numbers from 1 to 16, as shown in Table 4. The partner laboratories only had access to the protocol numbers, from 1 to 16.

The laboratories that participated in the present study provide services or carry out research on seed testing and are accredited by MAPA in different states of Brazil. They were chosen through acceptance of the invitation and logistical feasibility regarding rapid transfer of the samples and period for conducting tests and issuing results (Table 5).

The seed samples were packed in multilayer paper bags and placed in Styrofoam boxes for shipping to the participating laboratories (through carriers in land or air transport), together with the medium-size vermiculite to

Table 1. Codification, moisture content, and classification regarding sensitivity to the phytosanitary treatment of the soybean seed lots.

Cultivar	Moisture content (%)	Sensitivity	Lot
Cultivar 1	12.03	Sensitive	A
Cultivar 1	12.15	Sensitive	B
Cultivar 2	12.45	Not sensitive	C
Cultivar 2	12.34	Not sensitive	D

be used. The instructions for carrying out and evaluating the germination test in RP+V, as well as a questionnaire for evaluating the practical implementation of the methodology, were sent by e-mail.

Table 2. Composition of the standard slurry used in all the seed treatments.

Commercial product	a.i.	Class	Dose*
Maxim Advanced®	Thiabendazole (150 g L^{-1})	Fungicide	100 mL
	Fludioxonil (25 g L^{-1})	Fungicide	
	Metalaxyl-M (20 g L^{-1})	Fungicide	
Biocroma vermelho Biogrow®	-	Polymer	100 mL

*Recommendation for 100 kg of seed.

Table 3. Composition of the additional insecticide seed treatments.

Seed treatment	Commercial product	a.i.	Dose* (mL)	Final volume of the slurry** (mL)
Control	-	-	-	200
Thiamethoxam + cyantraniliprole	Cruiser 350® FS + Fortenza 600® FS	Thiamethoxam (350 $g.L^{1}$) + cyantraniliprole (600 $g.L^{-1}$)	200 + 60	460
Clothianidin + fipronil	Poncho® + Shelter®	Clothianidin (600 $g.L^{-1}$) + fipronil (250 $g.L^{-1}$)	100 + 200	500
Imidacloprid + thiodicarb	Cropstar®	Thiodicarb (450 $g.L^{-1}$) + imidacloprid (150 $g.L^{-1}$)	500	700

*Recommendation for 100 kg of seed.
**Final value including the base slurry with fungicide and polymer for 100 kg of seed.

Table 4. Identification of samples sent to the seed testing laboratory (STL).

Sample	Lot	Seed treatment
1	A	Control
2	A	Thiamethoxam + cyantraniliprole
3	A	Clothianidin + fipronil
4	A	Imidacloprid + thiodicarb
5	B	Control
6	B	Thiamethoxam + cyantraniliprole
7	B	Clothianidin + fipronil
8	B	Imidacloprid + thiodicarb
9	C	Control
10	C	Thiamethoxam + cyantraniliprole
11	C	Clothianidin + fipronil
12	C	Imidacloprid + thiodicarb
13	D	Control
14	D	Thiamethoxam + cyantraniliprole
15	D	Clothianidin + fipronil
16	D	Imidacloprid + thiodicarb

Table 5. Seed testing laboratories accredited by *Ministério da Agricultura e Pecuária* (MAPA), Brazil, participants in validation of the germination test in rolled paper plus vermiculite (RP+V) methodology for soybean seeds.

Seed Testing Laboratory	City/State
Agro Conecta Laboratório de Análises de Sementes e Diagnose de Plantas	Goiânia - GO
Agromen Sementes Agrícolas	Orlândia - SP
APROSMAT – Associação dos Produtores de Sementes de Mato Grosso	Rondonópolis - MT
Bayer CropScience LTDA	Uberlândia - MG
Laboratório de Análise de Sementes da Copercampos	Campos Novos - SC
Germinax - Análise e Certificação de Sementes	Formosa - GO
KWS Melhoramentos e Sementes LTDA	Patos de Minas - MG
Laboratório de Análise de Sementes e Mudas da UFLA	Lavras - MG
Laboratório Oficial de Análise de Sementes – LASO-MG/LANAGRO/Mapa	Belo Horizonte - MG
Seedcare Institute Syngenta	Holambra - SP
Sementest - Laboratório de Análise de Sementes	Ibiporã - PR
Syngenta Seeds LTDA	Uberlândia - MG
Vigor Agro Análises	Campo Belo - MG

In the protocol for implementation of the RP+V test sent to the laboratories, the methodology was as follows:

Germination in a rolled paper plus vermiculite (RP+V): eight replications of 50 seeds were sown on 2 paper towels damped with distilled water in the amount of 3 g of distilled water per g of paper towel, and a fine, uniform layer of medium vermiculite damped (55% to 95% of the particles > 2.4 mm, but the layer may also be composed of fine vermiculite – 65% to 95% of the particles > 1.2 mm) was added onto the already damped paper (2 sheets). The vermiculite had been moistened at the rate of 1 mL of distilled water: 1 g of vermiculite , in the amount of 100 mL of damped vermiculite per replication. After that, the 50 seeds of each replication were distributed with the aid of a seed counter and then covered with another sheet of damped paper. Finally, rolls were made, as is normally done in paper roll (RP) substrate tests. The rolls were kept in a Mangelsdorf germinator at 25 °C ± 2 °C. Normal seedlings, according Brasil (2009), were counted at seven days after setting up the test.

The laboratories were informed of the date for sending the samples, and they were asked to rapidly perform the test to avoid seed deterioration, as well as to immediately send the test results.

Step 3 – Statistical procedures and evaluation of results

After the samples were distributed and instructions were sent regarding the procedures to be carried out by the seed testing laboratories, the third step began. The results were sent to the authors and these results of validation were evaluated using the statistical methodologies in accordance with the Method Validation for Seed Testing (ISTA, 2006) and Rocha et al. (2023).

Statistical analysis: the data considered in the statistical analyses corresponded to the number of normal seedlings obtained in each sample, with evaluation of 400 seeds per sample conducted in eight replications of 50 seeds. The response variable under study was the percentage of normal seedlings observed in the samples sent to the laboratories. Statistical analyses were carried out using the R software (R Core Team, 2022).

Identification of outliers: to detect outliers in all the laboratories, the functional box-plot method (Sun and Genton, 2011) and the Hampel method (Huber, 1996) were used. The outliers detected were removed according to ISTA instructions (ISTA, 2006).

Evaluation of laboratory effects: analyses of variance (ANOVA) were carried out on the percentage of normal seedlings. In the analyses of variance, a 16 × 13 factorial arrangement was considered, composed of 16 samples [4 seed

lots (2 cultivars and 2 quality levels) × 4 chemical treatments] × 13 laboratories, in a completely randomized design.

Identification of outliers in the variances: Levene's test was carried out on each sample using the mean of normal seedlings of each sample per laboratory (ISTA, 2006).

Evaluation of accuracy (repeatability and reproducibility) and of Mandel's h and k statistics:

The repeatability variance (S^2_r) represents a measure of the variability within the laboratories (ISO 5725-2, 2019). With this value determined, the critical limit (*r*) of repeatability was calculated, estimated by $r = S_r D$, where D is obtained from the table of Tukey (1977), with degrees of freedom tending to the infinite and confidence level of 99% (Banzatto and Kronka, 2006). This *r* value was compared to the amplitude among the replications of each laboratory in each sample, thus evaluating which laboratories had acceptable repeatability, with a confidence level of 99%.

The reproducibility variance (S^2_R) represents a measure of the variability among and within the laboratories (ISO 5725-2, 2019), and with its value having been determined, the critical limit (*R*) of reproducibility was calculated for each sample, estimated by $R = S_R D$, where D is obtained from the table of Tukey (1977). This R value was compared to the range among the laboratories for each replication, thus determining which samples had acceptable reproducibility, with a 99% confidence level.

The precision and accuracy of the results of normal seedlings from each one of the laboratories was verified based on Mandel's *k* and *h* statistics and their respective critical values (Mandel, 1991). The *k* value, given by the ratio between the standard deviation of normal seedlings from each laboratory and the standard deviation of repeatability per lot, identified laboratories that showed differences among replications of normal seedlings above the critical level. The *h* value, in turn, indicated laboratories that overestimated (above the positive critical value) or underestimated (below the negative critical value) values of the percentage of normal seedlings in relation to the others.

RESULTS AND DISCUSSION

In initial characterization, differences were found among the seed lots regarding physiological quality in all the tests, except for germination (Table 6). In general, Lots A and D had statistically higher quality seeds than B and C, though with variations depending on the tests, such as vigor by AA, in which Lot A was superior, followed by D, with B and C statistically inferior to the others, without distinction between them (Table 6). Therefore, the study met the requirement of including at least three different levels of seed quality (ISTA, 2006).

An H value lower than zero was observed for all four lots (Table 7); considering the line corresponding to 8 (N) and the column for the properties of purity and germination/non-chaffy seeds in Table 1.2 of the RAS (Brasil, 2009), the critical value of H of 1.80 at 1% probability is obtained. As all the H values calculated were statistically lower than the critical H value listed, all the lots were considered non-heterogeneous, and thus it was possible to continue validation using all the lots.

Table 6. Results of the physiological tests before additional seed treatments.

Lot	FC	G	AA	ES
A	75 ab	82 a	65 a	91 a
B	69 b	74 a	34 c	84 ab
C	78 ab	78 a	32 c	76 b
D	83 a	86 a	57 b	90 a

*Mean values followed by the same letter in the column do not differ from each other by Tukey's test at 5% significance. FC: first count of RP germination test, G: germination in RP, AA: accelerated aging test, ES: emergence in substrate under controlled conditions.

Through the box-plots (Figure 2), possible discrepant values could be detected. The horizontal line in the center of the box indicated the median, the boxes represented the quartiles, and the vertical lines characterized the tails. To identify which of these points would in fact be considered outliers, the Hampel method was applied. The points represented possible values of normal seedlings that are candidates for outliers in each sample and laboratory. Only one outlier was observed and excluded in laboratory 12.

It is important to highlight that before conducting analysis of variance, the Shapiro-Wilk test was performed. The result was not significant ($p > 0.05$), indicating that assumption of normality for the residuals of the percentages of normal seedlings obtained from the RP+V methodology was satisfactory in all the samples evaluated.

Table 7. Results of the H test in different soybean seed lots.

Lot	H value	Condition	Recommendation
A	-0.6200	Homogeneous variances	Continue to analysis
B	-0.8000	Homogeneous variances	Continue to analysis
C	-0.4600	Homogeneous variances	Continue to analysis
D	-0.7400	Homogeneous variances	Continue to analysis

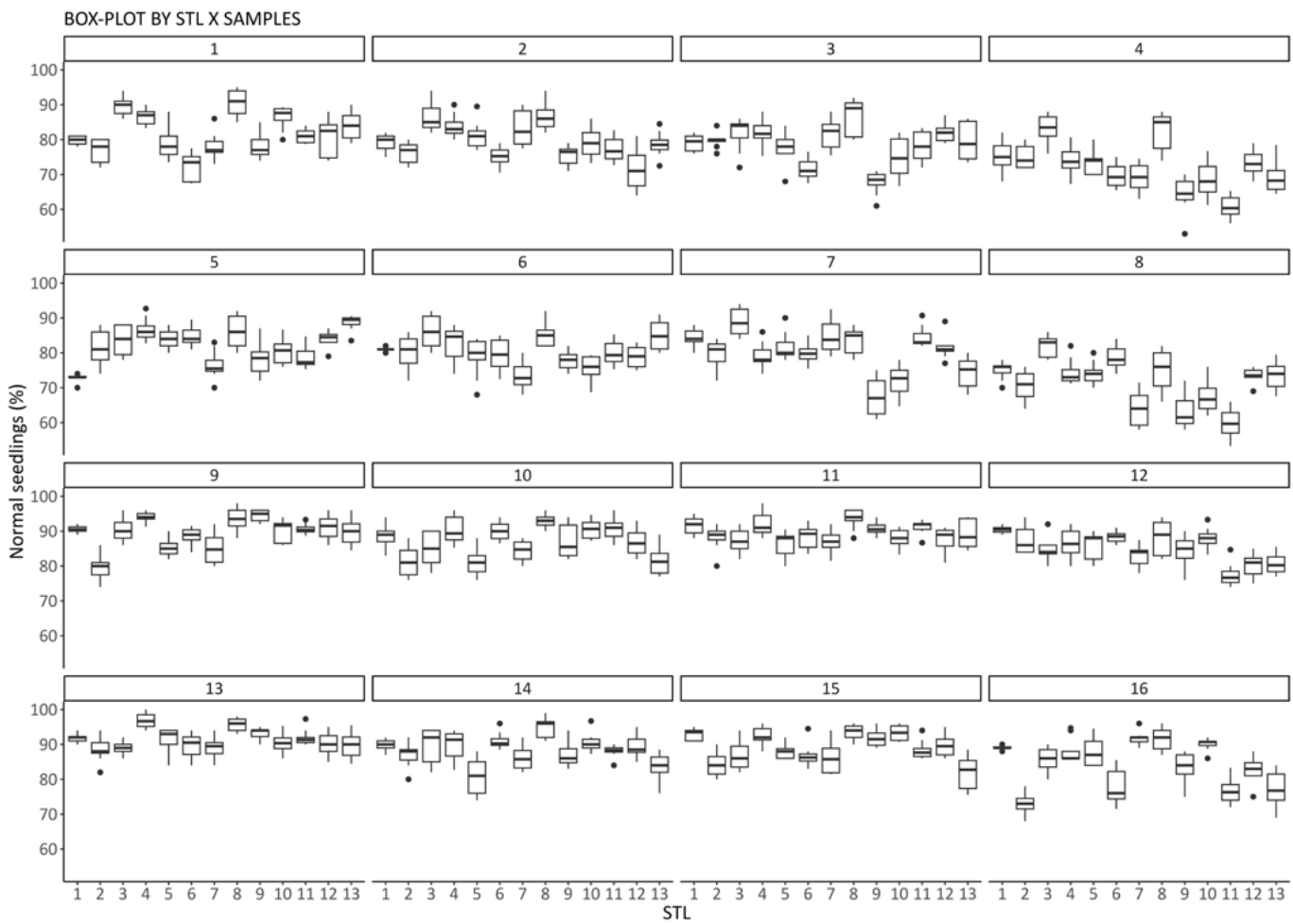

Figure 2. Box-plots of the results of the RP+V test of the soybean seeds treated and evaluated by analysts of each one of the 13 seed testing laboratories (STL) for the 16 samples evaluated.

Analysis of variance (ANOVA) was carried out considering the 16 × 13 factorial arrangement and the completely randomized design of the experiment. A significant difference was found both among the samples and among the laboratories, and there was also a significant interaction between them ($p < 0.01$).

There are significant differences in the sample factor, due to the use of different a.i. for phytosanitary treatments, different cultivars, and variations in the levels of physiological quality. Therefore, these differences were expected; furthermore, for validations, the ISTA requires inclusion of lots with at least three different levels of quality (ISTA, 2006). This was already observed in Table 6, where the lots exhibited differences in relation to vigor.

Differences among the laboratories may be related to the sensitivity of the F-test in large samples. That occurs because the F-test is a ratio between two variances and the variance decreases as the sample size increases. Consequently, the significance of the F-test becomes greater as the sample size grows, increasing the probability of rejecting the null hypothesis (Howell, 2012). Therefore, as the ANOVA was found to be significant for the laboratories, it was necessary to apply Levene's test to evaluate the homogeneity of the variances, a procedure recommended for large databases (Figure 1).

In Levene's test ($p < 0.01$), variabilities were found among the laboratories in each sample investigated (Table 8); however, this variation did not result in rejection of the equal variance hypothesis, thus allowing continuity of the analyses. This decision is important for minimizing the risk of mistakenly considering that a laboratory deviates from the standard when actually it does not.

The amplitude results from each laboratory (Lr_{ji}) and of each seed replication (Lr_{jk}), associated with critical limits and confidence levels of 99%, showed acceptable repeatability for all the laboratories and samples (Figure 3), which was also observed for reproducibility (Figure 4). For both repeatability (Figure 3) and reproducibility (Figure 4), a change was observed in the critical limit and in the variability of repeatability and reproducibility depending on the laboratories and the samples. However, all the values were below the critical limit, indicating the repeatability and reproducibility of the RP+V test for soybean seed testing.

Table 8. Result of Levene's test ($p < 0.01$) after removal of outliers by the Hampel test for detection of discrepant values in the variances for each sample of treated soybean seeds.

Sample	P value	Condition	Recommendation
1	0.2565	Homogeneous variances	Continue to analysis
2	0.2557	Homogeneous variances	Continue to analysis
3	0.1658	Homogeneous variances	Continue to analysis
4	0.8145	Homogeneous variances	Continue to analysis
5	0.0728	Homogeneous variances	Continue to analysis
6	0.0643	Homogeneous variances	Continue to analysis
7	0.6253	Homogeneous variances	Continue to analysis
8	0.1681	Homogeneous variances	Continue to analysis
9	0.0201	Homogeneous variances	Continue to analysis
10	0.2344	Homogeneous variances	Continue to analysis
11	0.6788	Homogeneous variances	Continue to analysis
12	0.2587	Homogeneous variances	Continue to analysis
13	0.3250	Homogeneous variances	Continue to analysis
14	0.2204	Homogeneous variances	Continue to analysis
15	0.1793	Homogeneous variances	Continue to analysis
16	0.1086	Homogeneous variances	Continue to analysis

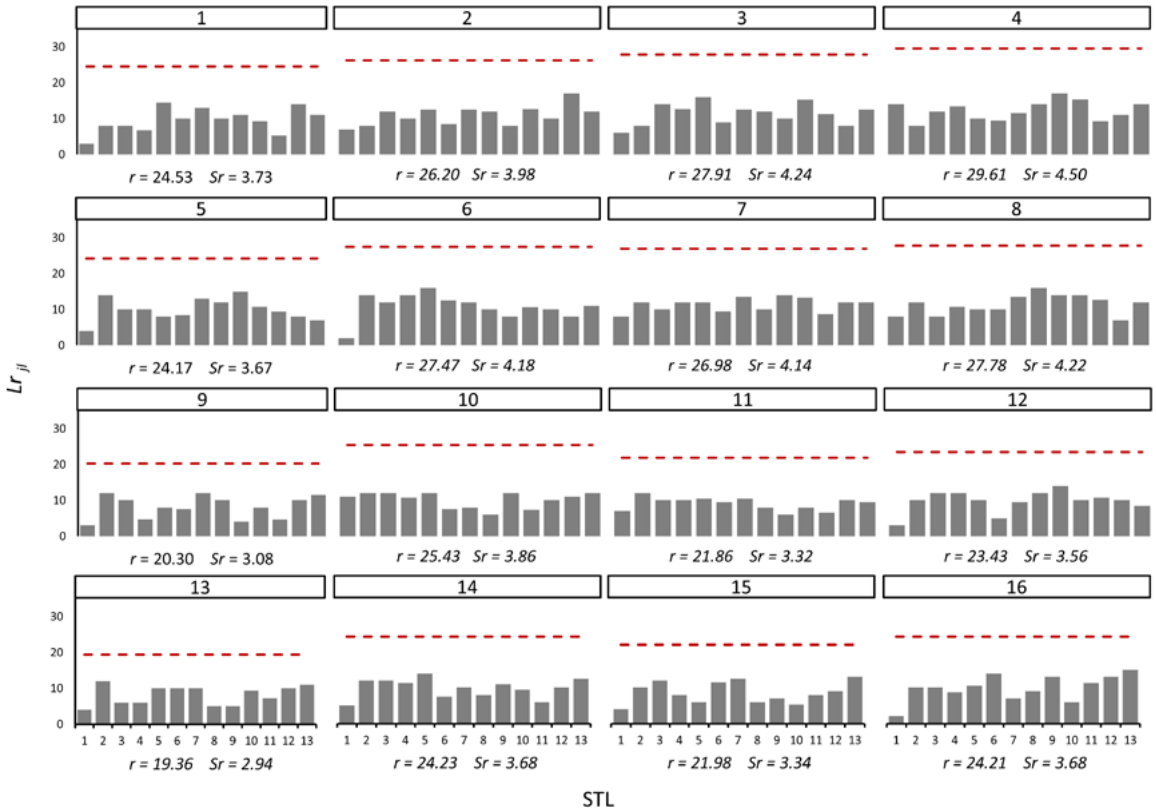

Figure 3. Width (Lr_{jl}), critical limit (r), and standard deviation of repeatability (Sr) for the 13 seed testing laboratories (STL) in each sample of germination test in a rolled paper plus vermiculite (RP+V) for treated soybean seeds.

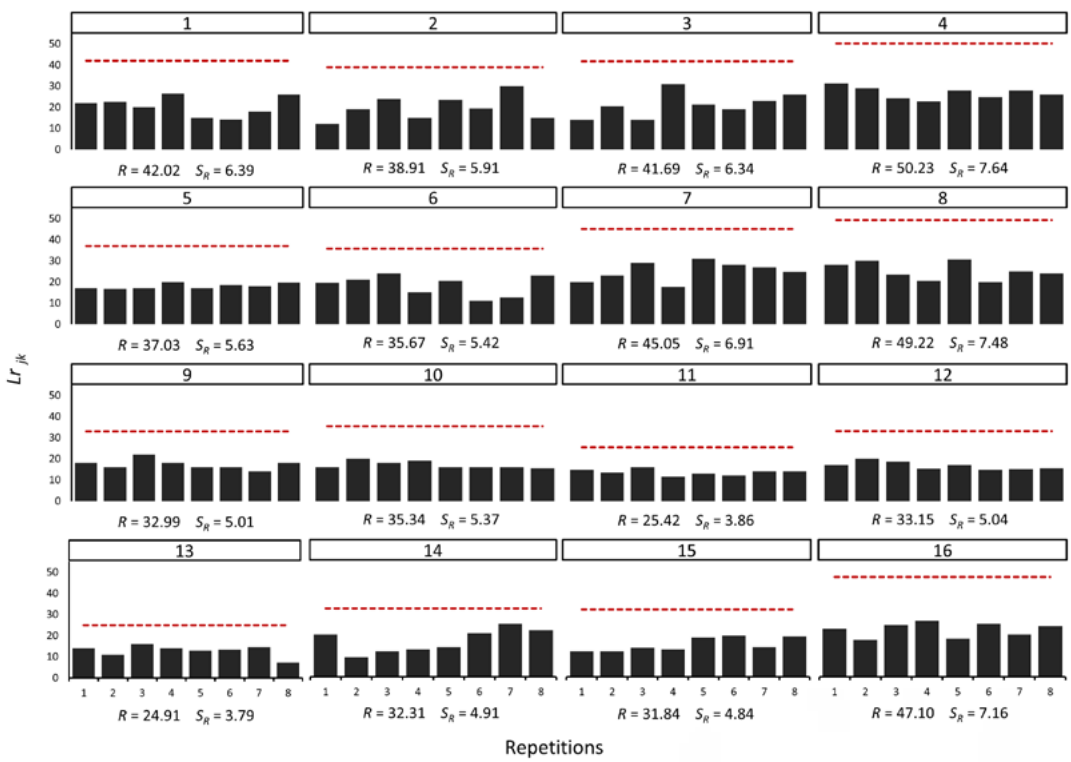

Figure 4. Width (Lr_{jk}), critical limit (R), and standard deviation of reproducibility (S_R) for the 8 replications in each sample of germination test in a rolled paper plus vermiculite (RP+V) for treated soybean seeds

Similar results of repeatability and reproducibility were described by Rocha et al. (2023) in a validation study of the germination test with vermiculite for treated maize seeds, with evaluation of 16 samples in 10 different laboratories.

The repeatability and reproducibility variances of each sample have different values, because they were affected by seed quality, since the lower quality lots frequently have wider variabilities, such as for samples 5, 6, 7, 8, 9, 10, 11, and 12. In general, lower variability suggests greater reliability of the results and better laboratory conditions for reproducing and repeating the proposed methodology (ISO 5725-2, 2019).

The graph of the *k* values for evaluating the accuracy of the results clearly shows there are various degrees of variability among the laboratories. Notably, laboratories 3, 5, 8, and 12 in two samples and laboratories 5 and 13 in one sample, highlighted in Figure 5, showed a trend toward the 1% critical limit (Figure 5). That suggests the presence of a random response among the laboratories, which, for its part, did not hurt the sample results, which indicated accuracy.

A random pattern in relation to overestimation and underestimation of the results considering Mandel's *h* statistic is shown in Figure 6. In this context, the mean accuracy of the samples was 58%, which was higher than that found by Rocha et al. (2023) in a similar study with maize, with 52% accuracy.

In spite of that, the present study showed that the tests were repeatable within the laboratories and were reproducible among different laboratories. Although some data showed degrees of precision and accuracy that exceeded the established critical limits of 1% and 5%, mentioned above, the results were generally consistent and reliable.

The variation in assessments of normal seedlings by analysts may be associated with individual experience in identifying abnormal characteristics caused by phytotoxicity, often resulting from treatment with certain a.i., as well as by the conditions and time for transporting samples to laboratories and time for carrying out analyses, which may also

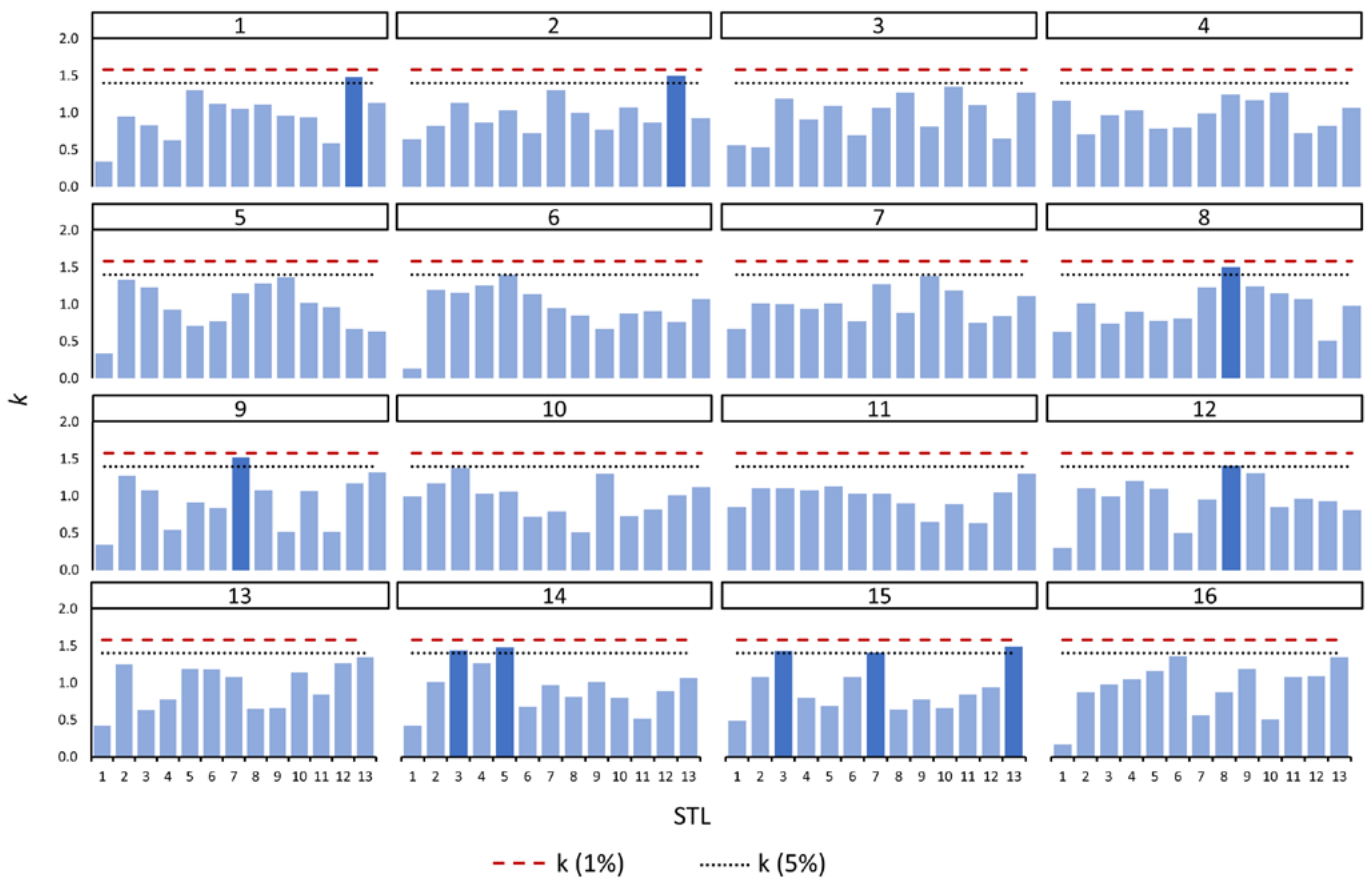

Figure 5. Graphs of *k* values for normal seedlings from treated soybean seeds in analyses made from the rolled paper plus vermiculite (RP+V) germination test for each sample in the 13 seed testing laboratories (STL).

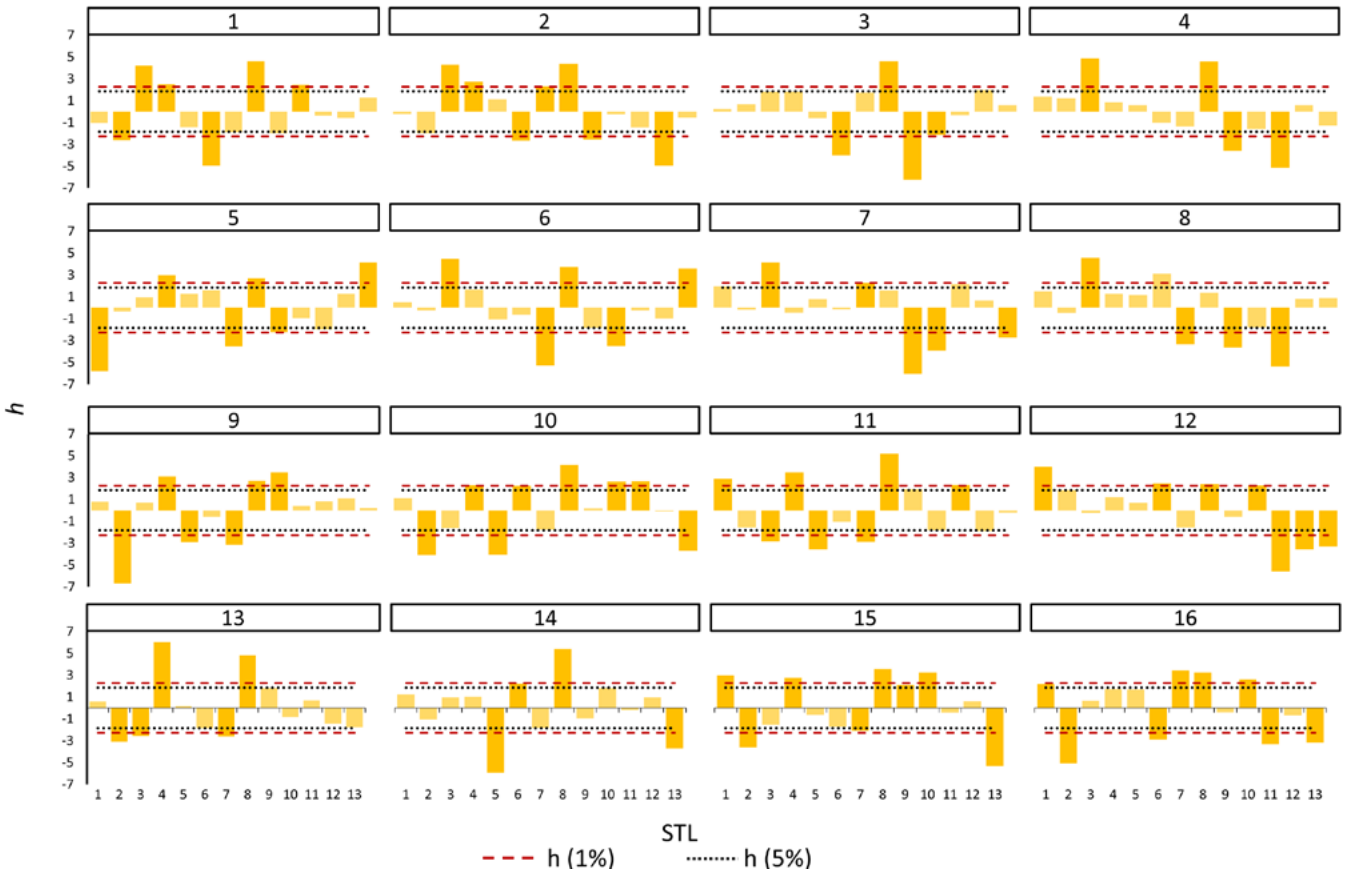

Figure 6. Graphs of *h* values for normal seedlings from treated soybean seeds in analyses made from the rolled paper plus vermiculite (RP+V) germination test for each sample in the 13 seed testing laboratories (STL).

have contributed to the simple effect of laboratories in the ANOVA. For Rocha et al. (2023), substantial differences in responses among analysts can lead to inconsistent results.

It is important to highlight that although variation was observed among different groups (analysts and laboratories) in Levene's test, homogeneity of the variances was achieved. That indicates that the laboratory variations in the germination test evaluations using the RP+V methodology are considered acceptable. Therefore, even with random variations in the results among analysts, the evaluation conditions remained within the acceptable levels of consistency. The results observed here were superior to those found in validation of germination in the RP+V method for the maize crop in the study of Rocha et al. (2023).

As we found that the methodology of germination using a RP+V showed consistency both in terms of repeatability and of reproducibility in all the laboratories tested, this methodology can be considered a viable option for evaluating germination of soybean seeds in the current context of production and quality control.

Considering the current scenario of the seed market, where the vast majority of seeds are sold with industrial seed treatment (IST), introducing new approaches that refine seed analysis is of utmost importance. Quality control in this field should accompany the progress that has occurred in the industry to meet the growing demands for quality, technology, and innovation in the seed market.

In the assessment questionnaire used together with this study, some laboratories reported that they already use the RP+V methodology as part of their internal quality control. Those that have not yet adopted this technique indicated the technical and operational possibility of incorporating it in their laboratory routine.

CONCLUSIONS

The data on normal soybean seedlings evaluated with the rolled paper with vermiculite (RP+V) methodology show repeatability, reproducibility, precision, and accuracy within the critical parameters of 1% and 5%.

The proposed methodology is reliable for use in a routine manner in evaluation of soybean seeds, above all for those treated with phytosanitary products.

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