ABRATES

Journal of Seed Science

ISSN 2317-1545
www.abrates.org.br/revista

*ARTICLE*

Journal of Seed Science, v.45, e202345026, 2023

http://dx.doi.org/10.1590/2317-1545v45274506

# Validation of the paper roll plus vermiculite (PR+V) germination test methodology for treated corn seeds

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**ABSTRACT**: A germination test is required by Brazilian standards for seed commercialization. The use of an adequate methodology that expresses the real physiological quality of seeds and minimizes possible phytotoxicity by chemicals is important for the production chain. In this context, the aim was to evaluate and validate the paper roll plus vermiculite (PR+V) germination test methodology for use in corn seeds. Two hybrids, one sensitive and the other tolerant to seed treatment, both with different vigor levels and under different chemical treatments, were used. Ten laboratories accredited by the *Ministério da Agricultura e Pecuária* (MAPA) were selected to perform and evaluate the PR+V germination tests. The data were subjected to statistical procedures to estimate the repeatability and reproducibility and check the accuracy and robustness of the test. The data were accurate, robust, and precise within the critical limits of 1 and 5%. The statistical results for repeatability, reproducibility, and bounds of the Mandel test were also accurate and precise at the critical limits of 1 and 5% for the normal seedlings evaluated by the PR+V methodology. Thus, the methodology can be used routinely to test corn seeds, especially those treated with phytosanitary products.

**Index terms:** chemical treatment, quality control, reproducibility, substrate, *Zea mays* L.

**RESUMO:** O teste de germinação é exigido pelas normas brasileiras para a comercialização de sementes. O uso de metodologia adequada, que expresse a real qualidade fisiológica das sementes e minimize possível fitotoxidez por produtos químicos é importante para a cadeia produtiva. Nesse contexto, objetivou-se avaliar e validar a metodologia de germinação rolo de papel mais vermiculita (RP+V) para uso em sementes de milho. Utilizou-se dois híbridos, sendo um sensível e outro tolerante ao tratamento de sementes, ambos com diferentes níveis de vigor e tratamentos químicos. Dez laboratórios credenciados pelo Ministério da Agricultura e Pecuária (MAPA) foram selecionados para realização e avaliação do teste de germinação RP+V. Os dados foram submetidos a procedimentos estatísticos para estimar a repetibilidade e a reprodutibilidade e verificar a precisão e robustez do teste. Os dados apresentaram acurácia, robustez e precisão dentro dos limites críticos de 1 e 5%. Os resultados estatísticos para repetibilidade, reprodutibilidade e padrões do teste de Mandel apresentaram exatidão e precisão que estão dentro dos limites críticos de 1 e 5% para plântulas normais avaliadas pela metodologia rolo de papel mais vermiculita. Assim, a metodologia pode ser usada rotineiramente para testar sementes de milho, principalmente para aquelas tratadas com produtos fitossanitários.

**Termos para indexação:** tratamento químico, controle de qualidade, reprodutibilidade, substrato, *Zea mays* L.

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**Received:** 04/05/2023.
**Accepted:** 06/27/2023.

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## INTRODUCTION

Seed quality is important for agribusiness, as it can affect the stand establishment, as well as the competitive ability of the plant, affecting its yield potential (Reis et al., 2022). Thus, this quality must be assessed accurately. Although the corn seed paper roll germination test methodology (Brasil, 2009) is used on a large scale by production companies, the use of seeds treated with phytosanitary products may underestimate the quality of a lot due to a greater possibility of phytotoxicity in this type of substrate. Thus, it may compromise the relationship of the results with the real physiological quality of the lot. Among the factors that can affect the test result are the chemical treatment of seeds and the substrate used in the test (Rocha et al., 2020; Moraes et al., 2022).

In Brazil, germination tests are performed in accordance with the Rules for Seed Testing, *Ministério da Agricultura e Pecuária* (Brasil, 2009). It is important to obtain comparable results between laboratories and minimize possible discrepancies between analyses within the production chain, among those who buy seeds, sell seeds, or supervise the process.

For technical and logistical reasons, chemical seed treatments are currently being performed before storage, mainly with the use of industrial seed treatment (IST) (Oliveira et al., 2020; Moraes et al., 2022). Despite the benefits associated with seed treatments, the storage of treated seeds can have deleterious effects on their germination potential, depending on the active ingredient used, among other factors (Carvalho et al., 2021). Currently, several products are used in the treatment of corn seeds, especially for IST, including fungicides and insecticides (Moraes et al., 2022). Today, most of the germination analyses performed in the seed production chain are with seeds treated with phytosanitary products.

Germination tests are performed to evaluate the physiological potential of seed lots under ideal conditions. However, with the corn substrates provided in the Rules for Seed Testing (Brasil, 2009), between sand or paper rolls, there are constant reports of inconsistencies in the results for treated seeds, probably related to phytotoxicity and the high concentration of active and/or rapidly absorbed ingredients (Rocha et al., 2020; Oliveira et al., 2020; Oliveira et al., 2021; Rossetti et al., 2021). Thus, adjustments to the methodology for conducting the tests would make them more accurate and representative, especially in terms of seeds treated with phytosanitary products.

The type of substrate used in a germination test may influence the results, depending on its structure, aeration, water retention capacity, and propensity to infestation by pathogens, among others, and may favor or hinder seed germination. The substrate establishes physical support for the seed and maintains the appropriate conditions for germination and seedling development (Martins et al., 2008). Thus, the type of substrate used must be appropriate to the physiological requirements of the germination and size and shape of seeds of each species (Brasil, 2009).

Among the substrates used for corn crop germination tests, paper roll and sand are officially indicated for use and analysis (Brasil, 2009). However, the use of vermiculite may be feasible and useful in some scenarios, especially for treated seeds. Vermiculite is a mineral similar to mica, formed essentially by hydrated aluminum and magnesium silicates. Its surface properties, in particular its surface area, hydrophobicity, porosity, and negative surface charge, make it recommended for use as an absorbent and carrier material (Ugarte et al., 2008). Therefore, in terms of germination tests, it can absorb concentrated chemical compounds.

The vermiculite that is suitable for seed analysis in laboratories has easily obtained characteristics, which are uniformity in chemical composition and particle size, porosity, water retention capacity, and a low density (Martins et al., 2008), in addition to its low cost compared to the price of germination papers and being a standardized product. With the use of this substrate in a test with treated soybean seeds, higher percentages of normal seedlings were observed compared to the substrates with greater water availability, due to the slower absorption of water and products during the imbibition process (Rocha et al., 2020). Bersch et al. (2021) also reported suitability of alternative methodologies, such as paper roll + sand and paper roll + vermiculite, for treated sweet corn seeds.

Due to the high use of corn seeds treated with phytosanitary products, research is needed to assist in adjusting and proposing new appropriate methodologies for evaluating these seeds to provide a greater correlation with the actual scenarios in the production fields and increased economic viability and to operationalize the methodology for seed

analysis in laboratories. The aim of this methodology validation was to develop a system that allows the use of new test methods through a comparison of equivalent tests and a review of existing methods to evaluate the quality of seeds that can be used by different laboratories that are safe, repeatable, and reproducible.

Thus, the aim of this study was to evaluate the germination methodology of paper roll plus vermiculite (PR+V) and validate its use for corn seeds.

## MATERIAL AND METHODS

The experiment was performed in three stages. In the first stage, two corn hybrids were selected, one with a greater sensitivity to the treatment of seeds with insecticides (TS) and the other with greater tolerance to the treatment with insecticides. For each hybrid, two lots with different vigor levels were selected for the initial evaluation. To determine the physiological quality of these corn lots, the following tests were performed in the laboratory:

*Germination between paper (BP):* with eight replications of 100 seeds, sown in germitest paper substrate (3 sheets of paper), in rolls, moistened with distilled water in an amount equivalent to 2.5 times the weight of the dry paper, which were kept in a Mangelsdorf germinator at 25 °C. The first germination count and the final germination count were performed. Normal seedling counts were performed at 4 and 7 days (Brasil, 2009).

*Germination in a paper roll plus vermiculite (PR+V):* with eight replications of 100 seeds, in rolls, moistened with distilled water in an amount equivalent to 3.0 times the weight of the dry paper. On the wet paper, a thin layer of moist vermiculite was added (vermiculite/distilled water ratio of 1:1), evenly distributed in an amount of 100 mL, and then sown with the aid of a seeding plate. Then, they were kept in a Mangelsdorf germinator at 25 °C (adapted from Rocha et al., 2020). Normal seedlings were counted at 4 and 7 days after sowing (Brasil, 2009).

*Germination between sand (BS):* with eight replications of 100 seeds, the seeds were placed in a tray with a white background and transparent plastic lid with internal dimensions of 307 x 132 x 115 mm and external dimensions of 353 x 178 x 121 mm on a uniform layer of sand with medium particle size. The seeds were covered with loose medium sand to obtain a layer of approximately 1 cm on the seeds. After sowing, the trays were covered. Subsequently, irrigation was performed in the sand until reaching 60% of its retention capacity (Brasil, 2009) and then the trays were maintained in a Mangelsdorf germinator. The evaluation was performed at 7 days, with the count of normally emerged seedlings.

*Cold test (CT):* This test was performed with four replications of 50 seeds sown in plastic trays with a substrate composed of sand + soil at a ratio of 2:1 and moistened to 60% of the water retention capacity. After sowing, the trays were placed in a cold chamber set at 10 °C without light and kept for 7 days. After incubation, the trays were transferred to a plant growth chamber regulated at 25 °C under a constant light regime, where they remained for 7 days. For the evaluation, the count of normally emerged seedlings was performed.

After the initial characterization of the different lots, in the second stage, the homogeneity was checked using the H test (Brasil, 2009), and those considered homogeneous were selected for the third stage; the heterogeneous lots were discarded (Table 1).

All lots were already treated with Maxin Advanced fungicide (Thiabendazole, Metalaxyl-M and Fludioxonil) and K-Obiol (Deltamethrin) and Actellic (Pirimiphos-methyl) insecticides, and additional industrial treatment was performed with insecticides, as shown in Table 2. The treatment process was performed in a Momesso Arktos Laboratory L5K machine for simulation of industrial treatment.

The combinations of the four lots with the four treatments of the additional seeds with insecticides were identified with numbers 1 to 16 to avoid any type of trend in the evaluation by the laboratories, as shown in Table 3. The participating laboratories only had access to the numbers of protocols identified as 1 to 16.

After conducting the treatment and packaging the seeds, they were stored in a cold chamber with controlled temperature (10 °C) and humidity (50%). The seed samples were sent to the ten participating laboratories for evaluation purposes (Table 4), all accredited by *Ministério da Agricultura e Pecuária* (MAPA). In addition to the seed samples, an

instruction protocol with the methodology, germination paper, and vermiculite to be used was sent to each laboratory. The laboratories performed the PR+V test, adapted from Rocha et al. (2020), as described above, with 8 replications of 50 seeds, and they sent the results (in percentages) for the normally emerged seedlings, abnormally damaged seedlings, abnormally infected seedlings, and dead seedlings separately by analyst.

Table 1. Hybrids selected according to the sensitivity to phytosanitary treatment and homogeneity.

| Hybrids | Sensitivity | Lot |
|---|---|---|
| Hybrid 1 | Not sensitive | Lot 1 |
| Hybrid 1 | Not sensitive | Lot 2 |
| Hybrid 2 | Sensitive | Lot 3 |
| Hybrid 2 | Sensitive | Lot 4 |

Table 2. Additional industrial seed treatments with insecticides.

| Commercial product | Active ingredient $g.L^{-1}$ | mL* | | |
|---|---|---|---|---|
| | | Dose | Polymer | Volume of spray solution |
| Cruiser 350 FS® | Thiamethoxam - 350 | 120 | 40 | 160 |
| Cropstar® | Imidacloprid - 150 + Thiodicarb - 450 | 350 | 40 | 390 |
| Poncho® | Clothianidin - 600 | 80 | 40 | 120 |
| Control | - | 0 | 0 | 0 |

*Recommendation for 60,000 seeds.

Table 3. Identification of samples sent to the laboratories.

| Samples | Description |
|---|---|
| 1 | Hybrid 1 lot 1 treated with Clothianidin |
| 2 | Hybrid 1 lot 1 treated with Thiodicarb + Imidacloprid |
| 3 | Hybrid 1 lot 1 treated with Thiamethoxam |
| 4 | Hybrid 1 lot 1 control |
| 5 | Hybrid 1 lot 2 treated with Clothianidin |
| 6 | Hybrid 1 lot 2 treated with Thiodicarb + Imidacloprid |
| 7 | Hybrid 1 lot 2 treated with Thiamethoxam |
| 8 | Hybrid 1 lot 2 control |
| 9 | Hybrid 2 lot 3 treated with Clothianidin |
| 10 | Hybrid 2 lot 3 treated with Thiodicarb + Imidacloprid |
| 11 | Hybrid 2 lot 3 treated with Thiamethoxam |
| 12 | Hybrid 2 lot 3 control |
| 13 | Hybrid 2 lot 4 treated with Clothianidin |
| 14 | Hybrid 2 lot 4 treated with Thiodicarb + Imidacloprid |
| 15 | Hybrid 2 lot 4 treated with Thiamethoxam |
| 16 | Hybrid 2 lot 4 control |

Table 4. Seed Testing Laboratory (STL) accredited by the *Ministério da Agricultura e Pecuária* (MAPA), Brazil, that performed the germination test in a paper roll plus vermiculite (PR+V) for corn seeds.

| Laboratories responsible | City/State |
|---|---|
| KWS Melhoramento e Sementes LTDA | Patos de Minas - MG |
| Laboratório de Análise de Sementes e Mudas – UFLA | Lavras - MG |
| Copercampos | Campos Novos - SC |
| Associação dos Produtores de Mato Grosso - APROSMAT | Rondonópolis - MT |
| LASO/LANAGRO – MG | Belo Horizonte - MG |
| Bayer do Brasil LTDA | Uberlândia - MG |
| Qualiteste Análises Agronômicas LTDA | Uberlândia - MG |
| Seedcare Institute Syngenta | Holambra - SP |
| Corteva Agriscience | Itumbiara - GO |
| Cotrijal | Não-me-toque - RS |

The physiological quality evaluations were also performed at the Seed Testing Laboratory (STL) of the Department of Agriculture (DAG), School of Agricultural Sciences of Lavras (ESAL), *Universidade Federal de Lavras* (UFLA), Lavras, MG, Brasil, through water content (Brasil, 2009), germination between paper (BP) (Brasil, 2009), germination between sand (BS) (Brasil, 2009), PR+V (adapted from Rocha et al., 2020), emergence (E) and cold test (CT) (Cicero and Vieira, 2020).

*Statistical analysis:* The data considered in the statistical analyses corresponded to the sum of normal seedlings obtained at each level (4 replications x 100 seeds), forming 400 seeds per level of vigor in the lots and per phytosanitary treatment. The response variable under study was the percentage of normal seedlings observed in the lots sent to the laboratories (Y). Statistical analyses were performed using R software (R Core Team, 2021).

*Lots profile:* analyses of variance of the physiological tests of the lots profile were performed, and their means were compared and grouped using the Tukey test at 5%.

*Outliers:* for the detection of conflicting values in all laboratories, the Hampel method was used (Hampel, 1974). A graphical representation of the boxplot chart (Tukey, 1977) was used to assist in the evaluation of the analyst's performance within each laboratory. Detected outliers were removed from the database before proceeding with the next analyses (ISTA, 2007).

Identification of outliers in the variances: the identification of outliers in the variances of the means at each vigor level was performed using Levene's test. The laboratories that showed higher variances due to the difficulty of matching the dates of analysis and interpretation were removed, and the process was repeated until only laboratories with homogeneous variances were present.

Evaluation of laboratory effects: analyses of variance (ANOVA) of the germination percentage were performed, and their means were compared and grouped using the Tukey test at 5%. In the ANOVA, a 16 x 10 factorial scheme was considered, i.e., 16 samples (combination of 2 levels of seed quality x 2 hybrids x 4 chemical treatment arrangements, as described in Table 3) x 10 laboratories according to the completely randomized design. Since the interaction between laboratories and the other factors was nonsignificant, it was considered negligible and was incorporated into the residuals.

Repeatability, reproducibility, and Mandel h and k statistics: the repeatability variation ($S^2r$) represents the variability within laboratories (ISO 5725-2, 1994). Once its value was determined, the critical limit (r) of repeatability was calculated by $r = S_{rj} D_j$, where $D_j$ was obtained from the Tukey table, with degrees of freedom tending to infinity and a confidence level of 99% (Banzatto and Kronka, 2006). The r value was compared with the amplitude between the replications of each laboratory at each level ($Lr_{jl}$) to indicate laboratories with acceptable repeatability.

The Mandel k statistic was used to evaluate the accuracy of the results, while the Mandel h statistic was used to graphically evaluate the estimate of bias and, therefore, the accuracy of the results (ISO 5725-2, 1994a). After the calculations, graphs of the Mandel h and k values were created for each level and laboratory and compared with the critical values for α = 1% and 5%.

## RESULTS AND DISCUSSION

The seed moisture content at the time of the tests did not differ statistically, with values ranging from 11.2% to 11.9%. This difference was of small magnitude, not exceeding 0.7% between the materials, in addition to being important for obtaining consistent results, since differences greater than 2% can interfere with the results of the physiological tests (Marcos-Filho, 1999).

Through the physiological tests performed before the additional seed treatment, it was found that the lots used met the minimum germination standard established for commercialization of corn seeds in Brazil (85%) (Brasil, 2013). Regarding vigor, there was a difference between lots in all tests, with lower values for lot 1 (Hybrid 1 – not sensitive) and lot 3 (Hybrid 2 - sensitive) than for the other lots. Therefore, the study met the assumption of different seed quality levels (Table 5).

Considering the row corresponding to 10 (N) and column for purity and germination attributes for non-clinging seeds, in Table 1.2 of the Rules for Seed Testing (Brasil, 2009), a tabulated value of 1.55 was obtained. When evaluating the values presented in Table 6 and considering the negative value as 0 (zero), it was observed that all values were lower than the tabulated value, and therefore, the lots under study were considered homogeneous by the H test.

Despite detecting variability between laboratories within each sample studied (Table 7), Levene's test ($p<0.01$) did not indicate a rejection of the hypothesis of homogeneous variances. This decision reduced the risk of considering a laboratory to be nonstandard, when in fact it was not.

Table 5. Results of the physiological tests before additional seed treatment.

| Lot | FC PR | G PR | FC PR+V | G PR+V | FC SG | SG | FC CT | F CT |
|---|---|---|---|---|---|---|---|---|
| 1 | 93 b | 96 a | 98 A | 98 a | 93 a | 96 A | 87 b | 87 b |
| 2 | 96 ab | 96 a | 98 A | 98 a | 96 a | 97 A | 97 a | 97 a |
| 3 | 66 c | 91 b | 76 B | 88 b | 71 b | 88 B | 75 c | 83 b |
| 4 | 99 a | 99 a | 99 A | 99 a | 97 a | 98 A | 96 ab | 100 a |

*Means followed by the same letter in the column do not differ by Tukey's test at 5% probability
FC PR: first count of the paper roll germination test, G PR: germination of the paper roll germination test, FC PR+V: first count of the paper roll plus vermiculite germination test, G PR+V: germination of the paper roll plus vermiculite, FC SG: first count of the sand germination test, SG: sand germination, FC CT: first count of the cold test, and F CT: final count of the cold test.

Table 6. Results of the Mandel h test of the different corn lots.

| Lot | h-value | Condition | Indication |
|---|---|---|---|
| 1 | -0.5199 | Homogeneous variances | Continuing the analysis |
| 2 | 1.2809 | Homogeneous variances | Continuing the analysis |
| 3 | 0.1732 | Homogeneous variances | Continuing the analysis |
| 4 | 0.2536 | Homogeneous variances | Continuing the analysis |

Table 7. Levene's test results (P < 0.01) after detecting and removing outliers in the variances for each sample of treated corn seeds.

| Sample | P-value | Condition | Indication |
|---|---|---|---|
| 1 | 0.3127 | Homogeneous variances | Continuing the analysis |
| 2 | 0.2527 | Homogeneous variances | Continuing the analysis |
| 3 | 0.4055 | Homogeneous variances | Continuing the analysis |
| 4 | 0.9159 | Homogeneous variances | Continuing the analysis |
| 5 | 0.9864 | Homogeneous variances | Continuing the analysis |
| 6 | 0.9083 | Homogeneous variances | Continuing the analysis |
| 7 | 0.2130 | Homogeneous variances | Continuing the analysis |
| 8 | 0.3631 | Homogeneous variances | Continuing the analysis |
| 9 | 0.0656 | Homogeneous variances | Continuing the analysis |
| 10 | 0.3905 | Homogeneous variances | Continuing the analysis |
| 11 | 0.1660 | Homogeneous variances | Continuing the analysis |
| 12 | 0.2927 | Homogeneous variances | Continuing the analysis |
| 13 | 0.2066 | Homogeneous variances | Continuing the analysis |
| 14 | 0.3390 | Homogeneous variances | Continuing the analysis |
| 15 | 0.9432 | Homogeneous variances | Continuing the analysis |
| 16 | 0.0733 | Homogeneous variances | Continuing the analysis |

There was a significant variation in the mean of the analyst observations for each of the 10 laboratories when considering the vigor level of each sample (Figure 1). In the box plots shown in Figure 1, there are candidate points for outliers. Hampel's method was used to confirm which of these points would be considered outliers, and those considered outliers were removed before the next statistical procedure, using Levene's test.

The ANOVA of the 16 x 10 factorial experiment, considering a completely randomized design, was conducted to check whether there was a significant difference between the 16 samples and between the 10 laboratories, as well as the interaction between them. There was a significant difference between the samples and between the laboratories and a significant interaction between them ($p < 0.01$). Kataoka et al. (2011) observed similar results regarding the validation of a germination test with radish, as observed by Wagner et al. (2016) in the validation of a method to shorten the controlled deterioration test for *Brassica* spp. by replacing the germination test with a conductivity measurement and França-Silva et al. (2019) for the implementation of radiographic analyses to evaluate infested corn seeds.

There were differences for the samples, as different products were used for the phytosanitary treatment, and different hybrids and vigor levels were also used. Therefore, differences were expected since, in the validation process, the principle is the use of lots or samples with different levels of quality (ISTA, 2007). Notably, before performing the ANOVA, the Shapiro–Wilk test was applied and was not significant ($p > 0.05$), showing that for the residuals of the percentage of normal seedlings obtained with the PR+V methodology at all levels, the assumption of normality was met.

When considering the use of the repeatability standard deviation, it was observed that sample 16, evaluated by laboratory-9, returned a nonnumerical result (not a number-NA), as shown in Table 8. A similar result was observed for the values of h for the comparison of variance of different groups, in which treatment 16 returned an NA in all laboratories. Often, in groups without variability, the quotient with a value close to zero in the denominator will tend to infinity, and the result will not be a number. Despite this result, the repeatability achieved in both groups assumed that the laboratory variances in relation to the PR+V methodology were equivalent.

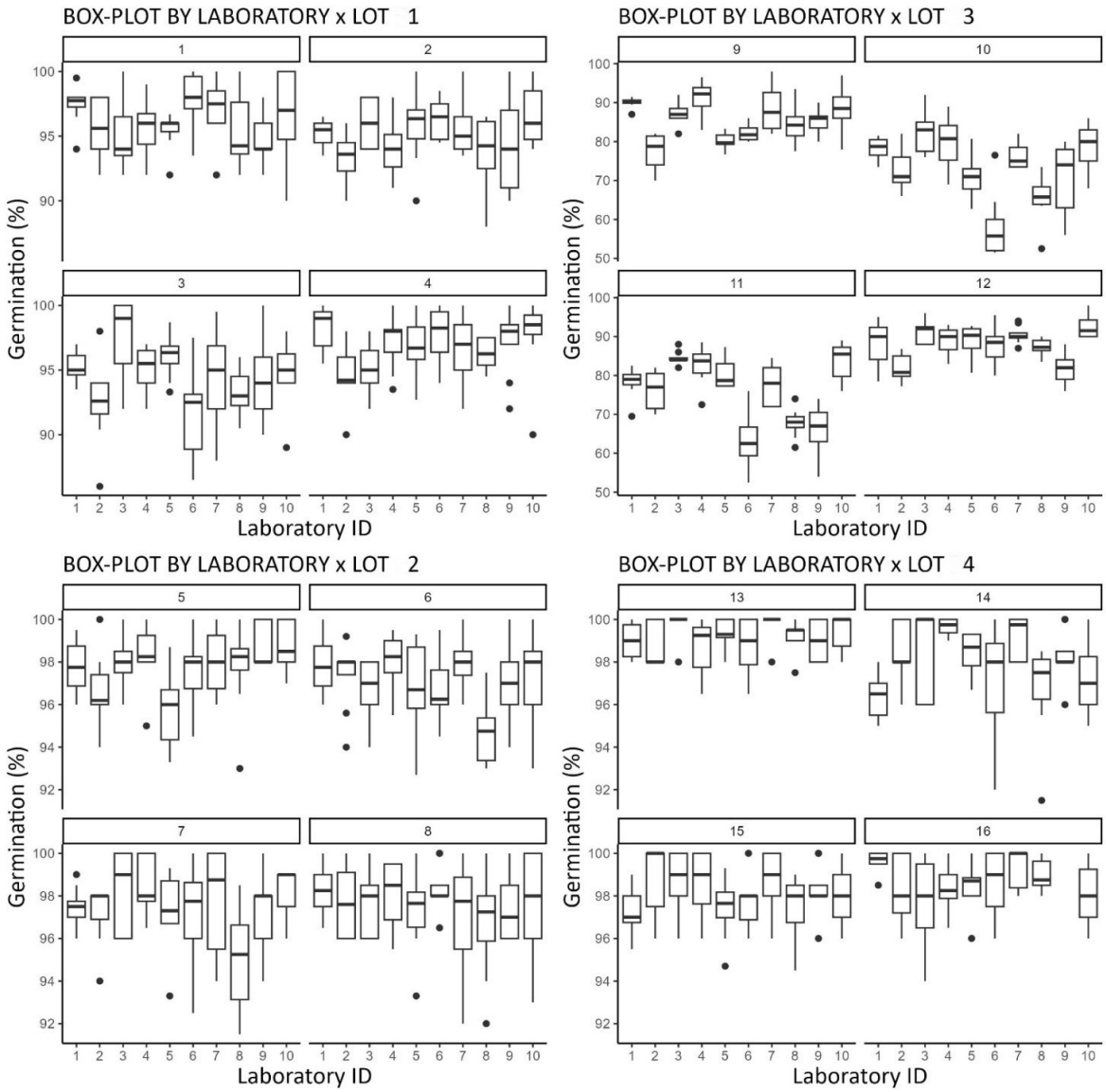


Figure 1. Germination test in a paper roll plus vermiculite (PR+V) for corn seeds treated and evaluated by analysts from each of the 10 Seed Testing Laboratories for the 16 samples evaluated. *Points represent the possible analyst values that are candidates for outliers for each hybrid and laboratory. The horizontal line in the center of the box indicates the median, the boxes represent the quartiles, and the vertical lines characterize the tails.

The amplitude results of each laboratory ($Lr_j$) and each seed sample ($Lr_{jk}$) (Table 9) associated with the critical limits and 99% confidence levels showed acceptable repeatability and reproducibility for all laboratories.

Both for repeatability (Table 8) and for reproducibility (Table 9), there was a change in the critical limit and in the variability as a function of the vigor levels and treatment used. The most sensitive hybrids to the treatment with insecticides and the most critical treatments affected the variability in the results.

Similar results of repeatability and reproducibility have been reported by Zecchinelli (2017) in a study on the introduction of *Brassica carinata* with a paper germination method at a constant temperature (20 °C) or alternating temperature (20-30 °C) and by França-Silva et al. (2019) in the study on corn seed infestation levels. It is likely that the repeatability and reproducibility variances were affected by seed quality, and low-quality lots often have larger variances. In general, the lower variability may indicate greater reliability of the results and suitable laboratory conditions for reproducing and repeating the proposed methodology (ISO 5725, 1994b).

Table 8. Width ($Lr_{jl}$), critical limit, repeatability standard deviation, and results of the determination of the repeatability (Re) condition (Cond) or not (Nr), critical limit (CL) for the laboratories in each analysis of germination in paper roll plus vermiculite (PR+V) for the treated corn seeds.

| A | LABS | | | | | | | | | | CL* | *SR* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $Lrj_1$ | $Lrj_2$ | $Lrj_3$ | $Lrj_4$ | $Lrj_5$ | $Lrj_6$ | $Lrj_7$ | $Lrj_8$ | $Lrj_9$ | $Lrj_{10}$ | | |
| | Cond | Cond | Cond | Cond | Cond | Cond | Cond | Cond | Cond | Cond | | |
| 1 | 5.5 *Re* | 6 *Re* | 8 *Re* | 7 Re | 4.7 Re | 6.5 Re | 8 Re | 8 Re | 6 Re | 10 Re | 16.4 | 2.5 |
| 2 | 3 *Re* | 6 *Re* | 4 *Re* | 7 *Re* | 10 *Re* | 4 *Re* | 6.5 *Re* | 8.5 *Re* | 10 *Re* | 6 *Re* | 16.1 | 2.5 |
| 3 | 3.5 *Re* | 12 *Re* | 8 *Re* | 5 *Re* | 5.4 *Re* | 11 *Re* | 11.5 *Re* | 5.5 *Re* | 10 *Re* | 9 *Re* | 18.3 | 2.8 |
| 4 | 4.5 *Re* | 8 *Re* | 6 *Re* | 6.5 *Re* | 7.3 *Re* | 6 *Re* | 8 *Re* | 3 *Re* | 8 Re | 10 Re | 15.7 | 2.4 |
| 5 | 3.5 *Re* | 6 *Re* | 4 *Re* | 5 Re | 5.4 Re | 5.5 Re | 4 Re | 7 Re | 2 Re | 3 Re | 10.3 | 1.6 |
| 6 | 4 *Re* | 5.2 *Re* | 4 *Re* | 4 *Re* | 6.6 *Re* | 5 *Re* | 4 *Re* | 4.5 *Re* | 6 *Re* | 7 *Re* | 11.1 | 1.7 |
| 7 | 3 *Re* | 4 *Re* | 4 *Re* | 3.5 *Re* | 6 *Re* | 7.5 *Re* | 6 *Re* | 7 *Re* | 6 *Re* | 3 *Re* | 12.5 | 1.9 |
| 8 | 3.5 *Re* | 4 *Re* | 4 *Re* | 4 *Re* | 6.7 *Re* | 3.5 *Re* | 8 *Re* | 8 *Re* | 4 Re | 7 Re | 13.4 | 2 |
| 9 | 4.5 *Re* | 12 *Re* | 10 *Re* | 13.5 Re | 6.6 Re | 6 Re | 16 Re | 16 Re | 10 Re | 19 Re | 28 | 4.2 |
| 10 | 8 *Re* | 16 *Re* | 16 *Re* | 20 *Re* | 18 *Re* | 25 *Re* | 8.5 *Re* | 21 *Re* | 24 *Re* | 18 *Re* | 41.7 | 6.3 |
| 11 | 13 *Re* | 12 *Re* | 6 *Re* | 16 *Re* | 10 *Re* | 23.5 *Re* | 12.5 *Re* | 12.5 *Re* | 20 *Re* | 13 *Re* | 32.6 | 5 |
| 12 | 16.5 *Re* | 9.6 *Re* | 8 *Re* | 10 *Re* | 12 *Re* | 15.5 *Re* | 7 *Re* | 6.5 *Re* | 12 Re | 8 Re | 24.9 | 3.8 |
| 13 | 2 *Re* | 2 *Re* | 2 *Re* | 3.5 Re | 2 Re | 3.5 Re | 2 Re | 2.5 Re | 2 Re | 2 Re | 6.36 | 1 |
| 14 | 3 *Re* | 4 *Re* | 4 *Re* | 1 *Re* | 2.6 *Re* | 8 *Re* | 2 *Re* | 7 *Re* | 4 *Re* | 5 *Re* | 10.9 | 1.7 |
| 15 | 3.5 *Re* | 4 *Re* | 4 *Re* | 4 *Re* | 4.6 *Re* | 4 *Re* | 4 *Re* | 4.5 *Re* | 4 *Re* | 4 *Re* | 9.55 | 1.5 |
| 16 | 1.5 *Re* | 4 *Re* | 6 *Re* | 3.5 *Re* | 4 *Re* | 4 *Re* | 2 *Re* | 2 *Re* | NA *Re* | 4 Re | 8.8 | 1.4 |

(*) Critical limits calculated by multiplying the repeatability values by a numerical Factor D, obtained from Tukey's (1977) Table, with degrees of freedom tending to infinity.

Table 9. Width ($Lr_{jl}$), critical limit, reproducibility standard deviation and results of the determination of the repeatability (Re) condition (Cond) or not (Nr), critical limit (CL) for the laboratories in each analysis of germination in paper roll plus vermiculite (PR+V) for the treated corn seeds.

| A | LAS | | | | | | | | CL | *SR* |
|---|---|---|---|---|---|---|---|---|---|---|
| | $Lrj_1$ | $Lrj_2$ | $Lrj_3$ | $Lrj_4$ | $Lrj_5$ | $Lrj_6$ | $Lrj_7$ | $Lrj_8$ | | |
| | Cond | Cond | Cond | Cond | Cond | Cond | Cond | Cond | | |
| 1 | 10 *Re* | 8 *Re* | 6 Re | 9 Re | 8 Re | 5.5 Re | 8 Re | 8 Re | 16.8 | 2.6 |
| 2 | 10 Re | 5 Re | 12 Re | 8 Re | 4.7 Re | 7.5 Re | 7.6 Re | 10 Re | 16.7 | 2.6 |
| 3 | 11 *Re* | 13 *Re* | 14 *Re* | 10 *Re* | 6.7 *Re* | 9.5 *Re* | 9.6 *Re* | 8.5 *Re* | 20.4 | 3.1 |
| 4 | 5.3 *Re* | 4 *Re* | 10 *Re* | 8 *Re* | 8 *Re* | 10 *Re* | 6.3 *Re* | 6 *Re* | 16.5 | 2.5 |
| 5 | 6 *Re* | 4 *Re* | 4 *Re* | 5.2 *Re* | 5 *Re* | 4 *Re* | 7 *Re* | 4 *Re* | 11.5 | 1.8 |
| 6 | 5 *Re* | 4.7 *Re* | 6 *Re* | 5 *Re* | 4 *Re* | 6.5 *Re* | 7 *Re* | 7.3 *Re* | 11.9 | 1.8 |
| 7 | 6.7 *Re* | 7.5 *Re* | 8 *Re* | 6.5 *Re* | 6 *Re* | 4.5 *Re* | 5 *Re* | 8.5 *Re* | 13.3 | 12 |
| 8 | 8 *Re* | 4 *Re* | 4 *Re* | 6.7 *Re* | 7 *Re* | 6 *Re* | 8 *Re* | 4 *Re* | 13 | 2 |
| 9 | 16.8 *Re* | 17.2 *Re* | 19 *Re* | 14.5 *Re* | 24 *Re* | 19 *Re* | 23 Re | 18 Re | 38.9 | 5.9 |
| 10 | 37 *Re* | 25.3 *Re* | 30 *Re* | 36 *Re* | 28.5 Re | 22.2 Re | 31 Re | 31.5 Re | 62.2 | 9.5 |
| 11 | 26 *Re* | 23.5 *Re* | 25 Re | 21.5 Re | 25 Re | 33 Re | 31.5 Re | 26.5 Re | 59.1 | 9 |
| 12 | 6.5 Re | 14.7 Re | 18 Re | 22 Re | 12.8 Re | 13 Re | 11.5 Re | 15 Re | 32.3 | 5 |
| 13 | 2 *Re* | 3 *Re* | 2 *Re* | 3.5 *Re* | 3.5 *Re* | 2 *Re* | 2 *Re* | 2.5 *Re* | 6.31 | 1 |
| 14 | 4.5 *Re* | 5 *Re* | 4 *Re* | 4 *Re* | 8.5 *Re* | 5.5 *Re* | 3 *Re* | 8 *Re* | 12.4 | 1.9 |
| 15 | 4 *Re* | 2.7 *Re* | 3 *Re* | 4.5 *Re* | 5.5 *Re* | 5.3 *Re* | 4 *Re* | 4 *Re* | 9.79 | 1.5 |
| 16 | 3.5 *Re* | 1.5 *Re* | 4 *Re* | 2 *Re* | 2 *Re* | 6 *Re* | 4 *Re* | 4 *Re* | 9 | 1.4 |

In the graph of the k values, different degrees of variability between laboratories were apparent, with laboratory-3 and laboratory-6 tending to reach a critical limit of 1% in relation to samples 16 and 14, respectively (Figure 2). A

random pattern was observed regarding overestimation and underestimation of the results for the Mandel h statistic (Figure 3), where laboratory-2 overestimated sample 15 and underestimated all other samples, exceeding the critical limits of 1 and 5%. This pattern was similar for laboratory-9, which overestimated sample 5 and underestimated all others (Figure 3).

Similar behaviors regarding the variability and levels of result underestimation and overestimation between laboratories were observed by Nijënstein and Eekelen (2015) in validating the germination of two species of the family Poaceae. Similarly, in a proposal to add chickpea (Fabaceae) to the conductivity test for seed vigor, Hosseini et al. (2014) found similar results.

França-Silva et al. (2019) also observed levels of result underestimation and overestimation for the infestation test using radiographs. In both validations, despite certain degrees of precision and accuracy above the established critical limits, on average, the tests were repeatable within laboratories and reproducible in different laboratories.

The variation in the responses of analysts in relation to the evaluation of normal seedlings may have been related to their experience in identifying seedlings with characteristics of abnormalities resulting from phytotoxicity commonly caused by treatment with some insecticides. As very different responses among analysts lead to incoherent results, it is notable that, although variation was found between the different groups (analysts and laboratories) in Levene's test, homoscedasticity was achieved, which indicates that laboratory variations in relation to the evaluation of the PR+V germination test were acceptable.

As the PR+V germination methodology was shown to be repeatable and reproducible in 100% of the laboratories, it is a viable option for evaluating the germination of corn seeds.

Evaluating abnormal and normal seedlings is facilitated with this methodology because, in comparison to the traditional methodology using only a paper roll, the vermiculite substrate in the PR+V methodology has properties

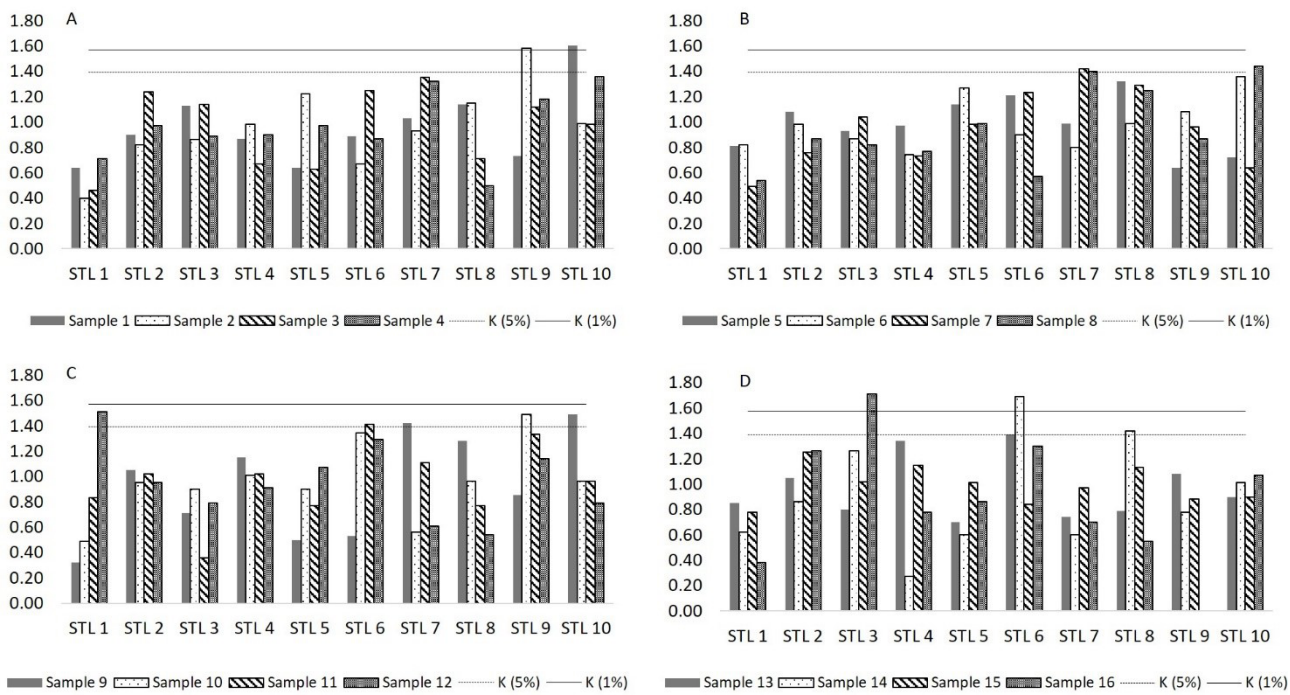


Figure 2. Graphs of k values for normal seedlings of treated corn seeds performed using the paper roll plus vermiculite germination test (PR+V). (A) Samples from hybrid 1 lot 1, (B) samples from hybrid 1 lot 2, (C) samples from hybrid 2 lot 3, and (D) samples from hybrid 2 lot 4, for the results of the 10 Seed Testing Laboratories (STL).

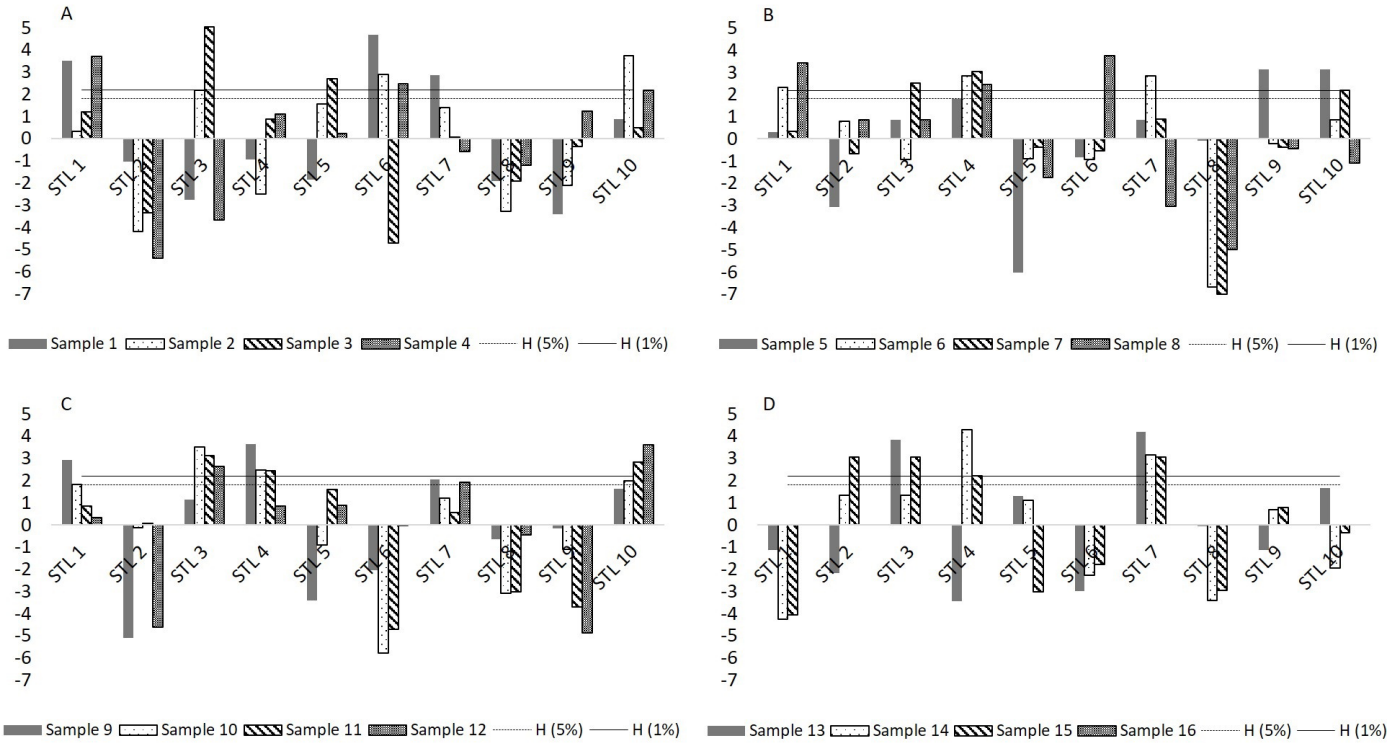


Figure 3. Graphs of the Mandel h values for normal seedlings of treated corn seeds using the paper roll plus vermiculite (PR+V) germination test. (A) Samples from hybrid 1 lot 1, (B) samples from hybrid 1 lot 2, (C) samples from hybrid 2 lot 3, and (D) samples from hybrid 2 lot 4, for the results of the 10 Seed Testing Laboratories.

that reduce the possible phytotoxicity problems caused by the products used in the treatment (Rocha et al., 2020). For Tunes et al. (2020), on paper roll the active ingredient remains concentrated around the seeds, which can lead to the known phytotoxic effect. Bersch et al. (2021) stated that the alternative substrates paper roll + sand and paper roll + vermiculite were promising to conduct the germination test on treated sweet corn seeds.

Additionally, these results may be related to the vermiculite causing a lower seed imbibition rate and, consequently, lower absorption level and contact with the active ingredients during the seed treatments. By restricting this absorption of water and chemical products, as occurs in the field/soil, this substrate alleviates the possible phytotoxic effects that the chemical products have on the development of seedlings in the paper roll. However, these hypotheses need to be evaluated in specific research.

The fact that vermiculite reduces the phytotoxic effects caused by germination in a paper roll was also clarified by Yang et al. (2018), who suggested that the occurrence of nonionic compound diffusion through the pericarp damages the endosperm, inhibiting the germination process and radicle formation. However, it is important to note that, even in the presence of the cutinized membrane, the active ingredients can pass through the endodermis during the process of seed imbibition (Baldini et al., 2018).

Rocha et al. (2020) showed phytotoxic effects on seedlings in germination methods with readily available water, such as in paper germination tests for treated soybean seeds, indicating that methods with slower initial imbibition, such as PR+V, are more appropriate for treated seeds.

Some analysts reported that they use the “between sand” method for treated seeds. However, the use of this approach requires more space in the laboratory for shelves, and the assembly and execution process takes more time since the sand must be sterilized before use. Other laboratories have claimed that they already use the PR+V methodology as internal quality control, and those that still do not use this technique report that it is possible to implement the

methodology in the laboratory. However, given the current seed market scenario, where most seeds are marketed with chemical phytosanitary treatments, it is extremely important to bring new techniques to aid in seed analysis.

As explained in this study, the use of vermiculite as a tool involves low operational complexity and is inexpensive, when compared to the traditional paper roll methodology. In addition, it provides greater safety and security for laboratories in the preparation of results and analysis of corn seeds, mainly treated ones. Quality control in seed analysis must follow the advances that have been implemented in the industry and thus meet the increasingly demanding market for seed quality, technology, and innovation.

## CONCLUSIONS

The statistical results for repeatability, reproducibility, and Mandel k and h values were accurate and precise, with critical limits of 1 and 5% for the normal seedlings evaluated by the PR+V methodology. It was possible to validate the proposed methodology because the data were accurate, robust, and precise within the critical limits of 0.01 and 0.05.

The proposed methodology, germination in a paper roll plus vermiculite (PR+V), can be used routinely to evaluate corn seeds, especially those treated with phytosanitary products.

## ACKNOWLEDGMENTS

The authors thank the *Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq, Fundação de Amparo à Pesquisa do Estado de Minas Gerais – FAPEMIG, Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (CAPES) and Seedcare Institute Syngenta for their financial support, scholarships granted and the Productivity in Research (CNPq) scholarship. Thanks also to the laboratories accredited by MAPA (*Ministério da Agricultura e Pecuária*) which participated in this proposed validation study.

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