Infrared thermographic assessment to discriminate soybean seed vigor

Article *in* Agronomy Journal · June 2022

DOI: 10.1002/agj2.21151

Francisco Guilhien Gomes-Junior
University of São Paulo

Agide Gimenez Marassi
University of São Paulo

Silvio Moure Cicero
University of São Paulo

Infrared thermographic assessment to discriminate soybean seed vigor

Francisco Guilhien Gomes-Junior [a, *], Agide Gimenez Marassi [b], Lívia Araújo Rohr [a] and Silvio Moure Cícero [a]

[a] Department of Crop Science, 'Luiz de Queiroz' College of Agriculture, University of Sao Paulo, CEP 13418-900, Piracicaba, SP, Brazil

[b] Physics Institute of Sao Carlos, University of Sao Paulo, CEP 13566-590, Sao Carlos, SP, Brazil

[*] Corresponding author.

E-mail address: francisco1@usp.br (F.G. Gomes-Junior).

ABSTRACT

The identification of differences in vigor among seed lots and individual seeds are important for research, seed industry and market. This study investigates the infrared thermographic assessment of soybean [*Glycine max* (L.) Merrill] seeds to discriminate seed lots based on their vigor levels. Seeds of five commercial lots were imbibed in moistened paper towel rolls (25 °C for 6 h) and thermographic measurements of average temperature, maximum temperature, minimum temperature, and thermal amplitude were performed after removing the seeds from the paper towel rolls and thermal stabilization in a controlled environment (25 °C and 70% relative humidity) for 15 minutes. The results indicated higher temperature levels from seeds in the lots with lower vigor than in those with higher vigor. The Pearson's

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/agj2.21151.

correlations from traditional tests and thermo measurements were higher for the average temperature on the seed surface. The infrared thermographic assessment is a promising protocol to discriminate seed lot vigor of soybean crops. This technique can be useful as a fast and non-invasive test in plant breeding and quality control programs of seed companies.

1. Introduction

A key component of the performance of crop seeds is the complex trait of seed vigor (Finch-Savage & Bassel, 2016). Seed vigor comprises the properties that determine the potential for rapid and uniform seedling emergence, in lots with acceptable germination, under a wide range of environmental conditions (ISTA, 2021). The major impacts of variation in seed vigor manifest through a negative direct effect on seedling emergence and therefore an indirect effect on yield (Marcos-Filho, 2015). How fast a seed germinates is an easy and recognizable indication of the concept of vigor (Finch-Savage & Bassel, 2016). These differences in vigor are directly related to variations in seed metabolism due to deterioration (Marcos-Filho, 2016).

Vigor tests are essential components of quality control of seed companies, as they can detect variations in the deterioration stage of seed lots even if they have similar germination (Baalbaki et al., 2009). Seed performance after sowing or during storage validates whether the potential identified by the vigor tests was reached and how suitable were the procedures used for this assessment (Marcos-Filho, 2015). Thus, seed quality control must include efficient tests that identify, quickly and accurately, differences in vigor among seed lots (Fessel et al., 2010). Traditional procedures, such as electrical conductivity, seedling growth, accelerated aging and tetrazolium tests have been recommended to assess the vigor of soybean seeds (Vieira et al., 2003). However, those methods are usually non-automated, time-consuming, destructive, and require specialized training and experience. Therefore, non-invasive and high-throughput screening methods are needed for the seed industry to provide high-vigor seeds for farmers before sowing (Xia et al., 2019).

In recent decades there has been a significant advance in computer science and electronic technologies which can benefit the seed vigor analysis. In soybean, one example is the digital image processing and artificial neural network to evaluate seed vigor based on seedling growth measurements (Hoffmaster et al., 2003; Rodrigues et al., 2020). However, most studies with different species, based on non-destructive techniques, such as near infrared spectroscopy, multi- and hyperspectral images, Raman spectroscopy, infrared thermography, and soft X-ray imaging have been focused on the detection of seed viability instead of seed vigor (Xia et al., 2019). Among those imaging techniques, infrared thermography can be very useful for ranking seed lots based on vigor levels.

Infrared thermography is a non-invasive method that quantifies the surface temperature of bodies by capturing the emitted thermal radiation, generating a digital image called a thermogram (Lahiri et al., 2012). Thermography systems differ from other systems with remote sensors because they measure emitted radiation whereas optical systems measure the reflected radiation (Mastrodimos et al., 2019). Thermal sensors have been used to estimate seed viability as a good alternative to conventional methods (ElMasry et al., 2020). Infrared thermography studies in pea seeds revealed lower surface temperature in higher viable seeds than in less viable ones (Baranowski et al., 2003; Men et al., 2017) and allowed a real time visualization of the earliest physic-chemical events of germination and diagnose seed viability long before the primary root emergence (Kranner et al., 2010). Studies with lettuce (Kim et al., 2013) and pepper seeds (Kim et al., 2014) demonstrated the efficiency of pulsed thermographic analysis to discriminate artificially aged and healthy seeds in terms of lifetime thermal decay.

More recently, based on thermal decay and regression model, the infrared thermography was demonstrated as a promising non-invasive examination of seed vigor (Liu et al., 2020). Considering that more deteriorated seeds present more damaged cell membranes and, consequently, with greater permeability when compared to less deteriorated ones (Marcos-Filho, 2016), the applicability of the infrared thermography for vigor assessment would be related to the detection of differences in metabolism between seeds considering variations in the water absorption rate. The variation of seed heat flow has been proved to be affected by water imbibition, respiration, decomposition of nutrients and other biochemical, physical, chemical reactions correlated with seed viability during the germination processes (Kim et al., 2013; Kim et al., 2014). Thus, with the detection of differences in the thermodynamic properties of seed tissues, it is possible to discriminate the lots in terms of their physiological potential. In this study, we applied infrared thermography to detect thermal variations in the surface of soybean seeds and evaluated the efficiency of this technique to discriminate seed lots according to their vigor levels.

2. Materials and Methods

2.1 Seeds

Five commercial seed lots of soybean [*Glycine max* (L.) Merril], cv BMX Potência RR with moisture contents from 9 to 10% (wet basis), determined by the oven method at 105 °C for 24 h (Brasil, 2009) were investigated in this research.

2.2 Seed germination and vigor tests

The germination rate (GER) was carried out using five replications of 50 seeds from each seed lot, using paper towel rolls moistened with the quantity of water weighing 2.5

times the dry weight of the substrate at 25 °C. The percentages of normal seedlings were calculated at four (first count - FC) and seven days; test interpretation was based on criteria in the Rules for Seed Testing (Brasil, 2009). Moreover, vigor tests comprised the accelerated aging test (AA), field seedling emergence (SE), electrical conductivity (EC), seedling length (SL) and the uniformity of seedling development (UNF).

The AA test using a plastic box (11 cm × 11 cm × 3.5 cm) had the seed samples from each lot distributed in a single layer on a wire mesh above the liquid containing 40 mL of saturated NaCl solution to provide an environment with relative humidity of 76% (Jianhua & McDonald, 1996). The plastic boxes were held in a water jacketed accelerated aging chamber at 41 °C for 48 h, and thereafter germination was performed in five replications of 50 seeds from each lot. The records were percentages of normal seedlings for each lot.

The SE test was conducted in five replications of 50 seeds sowed under lines of 4 m, spaced 0.4 m apart, in which seeds were distributed at uniform distance, in a depth of approximately 0.03 m. Spray irrigation was done according to need and, at the end of 14 days, the percentage of seedling emergence was calculated to obtain mean values for each lot.

The EC was evaluated using the bulk method with five replications of fifty seeds for each seed lot. The seeds were weighed on an analytical balance with 0.01 g precision, immersed in 75 mL of deionized water into plastic cups (200 mL) and held in a germination chamber at 25 °C for 24 h. Thereafter, the electrical conductivity of the soaking solutions was determined using a conductivity meter (Digimed, DM-32). Evaluation was made in a temperature-controlled environment at 25 °C and the device was connected to constant electric current. The results obtained were divided by the mass of the fifty seeds and expressed in $\mu S\ cm^{-1}\ g^{-1}$ of seeds (Baalbaki et al., 2009).

The SL and UNF evaluations were carried out using the Automated Seed Vigor Analysis System - Vigor-S (Rodrigues et al. 2020). Five replications of 20 seeds per lot were distributed in two rows of 10 seeds in the upper third of two sheets of paper towel and covered with a third sheet. The substrate was previously moistened with a quantity of water weighing 2.5 times the dry weight of the substrate. The rolls containing the seeds were germinated at 25 ± 1 °C for three days. Next, the seedlings of each replication were transferred to a sheet of navy-blue Ethylene-vinyl acetate (EVA), and digitalized on a scanner (HP, Scanjet 200), fixed upside-down inside an aluminum box (60 cm × 50 cm × 12 cm), resolution of 300 dpi. The images were processed by the Vigor-S software and the average seedling length (cm) and the seedlings development uniformity index (from 0 to 1000) for each lot were obtained.

2.3 Thermographic measurements of seeds

Seeds in groups of 50 were placed in paper towel rolls moistened with a quantity of water weighing 2.5 times the dry weight of the substrate and held in a germinator at 25 °C for 6 h. Next, digital thermal images were obtained using a FLIR T650sc camera (FLIR Systems Inc., USA) with a detection spectrum of 7.5−13 µm. This infrared system has an image resolution of 640 x 480 pixels and thermal sensitivity less than 0.020 °C (< 20 mK) at 30 °C. Seed temperature was homogenized before thermographic measurements by removing the seeds from the paper towel rolls and transferring them to a sheet of black EVA (11 cm x 11 cm) held under controlled environment (25 °C and 70% relative humidity) for 15 minutes. The thermography imager was positioned from a distance of 50 cm of the seeds. Qualitative maps of temperature (thermograms) and quantitative data were obtained from the surface of seeds.

The digital thermograms were analyzed by FLIR ResearchIR Max software (FLIR Systems Inc., USA) and the results were displayed using the "rainbow" palette, with white/red as hot and blue/black as cold. After obtaining the qualitative thermal images, regions of interest (ROI) were delineated in each seed using the circle tool embedded in the FLIR ResearchIR Max software. Thermal measurements were performed in ~~200~~ seeds from each lot. The thermal data of the pixels within the ROI were calculated to estimate the maximum temperature (Tmax), minimum temperature (Tmin), average temperature (Tavg) and thermal amplitude (Tampl) of each seed. These thermographic data were used to identify differences among seed lots, and correlate them with other tests recommended for assessing the vigor of soybean seeds. The thermographic measurement procedure is illustrated in Figure 1, and includes the representation of seedlings from a higher and lower vigor lot. After the thermal images, color reflectance red-green-blue (RGB) images were obtained using a digital camera with a 12-megapixel resolution CCD sensor.

2.4 Statistical analysis

Seed germination and vigor data were submitted to the analysis of variance (ANOVA) in a completely randomized design, except for the data of seedling emergence in the field, which were analyzed according to the randomized block design. The data obtained were submitted to presuppositions based on normality and homogeneity of residual variances. The Shapiro-Wilk normality test showed that data were normally distributed and mean values were grouped by the Fisher's least significant difference [LSD] test ($P \leq 0.05$). The correlation among the variables were tested by the Pearson's linear correlation coefficient (r). The correlation coefficients (ρ) were considered significant at 5% probability by the T test. Principal component analysis was performed using all parameters analyzed. Data analysis was performed using statistical software R 4.0.5 (R Core Team, 2019).

3. Results and Discussion

The objective of seed vigor tests is to provide the identification of important differences in physiological potential among seed lots to identify lots with higher probability of performing well after sowing, during the storage, or both (Marcos-Filho, 2015). Based on the ANOVA, significant differences were found among seed lots for germination and vigor tests (Table 1).

Results of FC, AA, SE, EC and SL indicated the seed lot 1 as the less vigorous. The EC test revealed differences between lot 1 and 3 greater than 42 µS cm^{-1} g^{-1}. Research carried out with soybean seeds confirmed the efficiency of the EC for separating seed lots into different vigor levels: very high - for lots that have EC ≤ 70 µS cm^{-1} g^{-1}; high - for lots with EC from 71 to 90 µS cm^{-1} g^{-1}; medium - lots that have EC from 91 to 110 µS cm^{-1} g^{-1}; and low - lots that have EC ≥ 111 µS cm^{-1} g^{-1} (Prado et al., 2019). Based on these categories, seed lots 2 to 5 were classified as high vigor levels and lot 1 as low vigor level.

It is important to consider that only one test cannot detect all the possible facets of seed performance, and the information provided about seed vigor should be based on the results of two or three tests whose principles might be closely related to desirable objectives (Marcos-Filho, 2015), such as selection of seed lots with greater potential for seedling emergence in the field. Thus, seed lots 3 and 4 showed the highest percentage of normal seedlings detected by the AA test (89% and 88%, respectively) and the largest seedling lengths (7.4 and 7.7 cm, respectively). Moreover, it should be emphasized that all the lots exceeded the minimum germination established for the soybean seed trade in Brazil (G ≥ 80%). This is important because it would not be consistent to evaluate the vigor of soybean seed lots with germination lower than 80%, that is, evaluation of seeds not suitable for sowing in commercial fields (Rodrigues et al., 2020).

This study aimed to evaluated the efficiency of thermographic assessment in raking seed lots into vigor levels in comparison to traditional vigor tests. The results of Tmax, Tmin, Tampl (Tmax − Tmin) and Tavg estimated from each pixel inside the ROI of seeds are shown in Figure 2. The seeds showed variations on their surface temperature calculated from the thermal images obtained after 6 h of imbibition at 25 °C. Box-and-whisker plots showed large variability of data for the five seed lots. However, the lowest variability was observed for Tmax in seeds from lot 1 compared to the others lots, and for Tmin and Tavg in seeds from lots 2, 3 and 4 compared to lots 1 and 5. Based on the mean comparisons (LSD test, $P \leq 0.05$), the seeds from lot 1 showed the highest Tmax (20.6 °C; Figure 2A), and the seeds from lots 3 and 4 the lowest values (19.9 and 19.8 °C, respectively). Similar results occurred for the Tmin (Figure 2B), which seeds from lot 1 showed higher values than the other seed lots. Likewise, the Tavg from lot 1 (19.9 °C) was higher than other seed lots by up to 0.7 °C (lot 1 versus lot 4; Figure 2C). Regarding to Tampl (Figure 2D), seeds from lot 2 showed the highest value (1.1 °C) when compared to lots 3, 4 and 5.

Although all the thermal parameters identified differences among the seed lots, the measurement of the Tmax and Tavg discriminated the soybean seed lots similarly to tests traditionally used to vigor assessment (Table 2). The less vigorous lots had a higher Tmax and Tavg in relation to the more vigorous ones, as it can be seen by comparing lot 1 (lowest vigor) with lots 3 and 4 (highest vigor). These results corroborate the study by Baranowski et al. (2003) in pea seeds, in which viable seeds showed a considerable reduction in the average temperature (more than 1 °C) in relation to non-viable seeds during the first 12 h of imbibition. These differences in temperature after 6 h of imbibition may be related to variations in the internal metabolic activity of soybean seeds depending on their vigor level.

According to ElMasry et al. (2020), the viable seeds stay at this cool temperature because the storage reserves continuously break down into sugar, whereas certain biophysical properties of the aged seeds affect the speed of water uptake and fail to break down the food reserves delaying the thermal profile. Furthermore, respiration can also be a process that leads to water production and heat release, which is affected by the seeds' vigor level (Liu et al., 2020). Previous research with pea seeds also revealed that the non-viable seeds had the fastest temperature rise while the viable seeds had the slowest, and these differences mainly occurred in the first 3 h of imbibition (Men et al., 2017).

Based on the visual analysis of the RGB image, differences in color between seeds after the imbibition were not easily detectable (Figure 3A). On the other hand, the "rainbow" color palette from the thermograms allowed to identify differences in the surface temperature of seeds from different lots and between seeds of the same lot (Figure 3B). Most of the seeds from lot 1 showed predominantly green, yellow and orange colors (indicating higher surface temperatures of ~ 20 °C) while for seeds from lot 3, the surface temperature was lower (~ 19 °C), as evidenced by the predominant blue and purple colors. As an example, a comparative analysis between the seed 10 (lot 1) and seed 8 (lot 3), highlighted in Figure 3B, shows that the Tmax, Tmin and Tavg of the seed 10 were higher than the seed 8 at 1.7, 1.6 and 1.9 °C, respectively (see table imbibed in Figure 3B). In comparisons performed within each seed lot, and using lot 1 as an example, the Tmax, Tmin and Tavg between the seeds 1 and 10 varied by 0.4, 0.6 and 0.6 °C, respectively. The examination of Figure 3B shows that similar behavior also occurred among seeds from the other lots.

The thermal variations among seeds from the same lot indicate the heterogeneity in terms of vigor for individual seeds in a population. According to Marcos-Filho (2016) a seed lot discriminated as high vigor is never composed exclusively of high vigor seeds, but of seeds with varying physiological levels although with predominance of vigorous seeds. This same thought applies to a low vigor lot, which is not a hundred percent composed of low vigor seeds. Hence, it was confirmed here that infrared thermography can be a very useful tool for the analysis of individual seeds, allowing the non-destructive identification of less vigorous seeds in commercial soybean seed lots. Most of the methods used to assess seed vigor provide information on the general quality of seed lots without referring to variations of individual seeds.

The results of Pearson's correlations ($p \leq 0.05$) between the evaluations of physiological potential and thermographic measurements of the seeds revealed the highest values for Tavg (Figure 4A). Negative correlation was observed between Tavg and physiological parameters of FC, SL, AA, GER and SE, with coefficients of $r = -0.85$, −0.83, −0.8, −0.77 and −0.73, respectively. The EC test showed a positive correlation with the Tavg ($r = 0.83$), which was suitable since the EC measurement is inversely proportional to seed vigor and the thermography data showed higher Tavg in seeds with lower vigor. A biplot using the first two principal components (PC) was built to separate the seed lots based on evaluations of seed physiological potential and thermography (Figure 4B). Principal components PC1 and PC2 explained 90.0% and 5.2% of the variation among lots, respectively. There was a clear spatial separation of the seed lots and evaluations of FC, AA, SL, Tavg SE, GER, Tmax and Tmin were the most important to explain the data variability in the PC1. The PC2 was mainly correlated with Tampl, EC and UNF. The proximity of lots 3 and 4 with the variables GER, AA, FC, SL and SE evidences their greater vigor when compared with lot 1, which was positioned in the opposite direction and close to the EC, Tmim and Tavg variables.

There are two basic configurations to build up an infrared thermographic imaging system in seed quality monitoring: the passive thermal imaging and the active thermal imaging approach. Passive-based system is normally used to describe the surface thermal properties of the objects under investigation and for non-contact temperature measurement during processing (ElMasry et al., 2020). In the present study, the thermal measurements of soybean seeds were acquired by using a passive thermal imaging configuration, with the heat been generated naturally by the tested seeds after a period of 15 minutes of thermal stabilization in a controlled environment at 25 °C and 75% relative humidity. This procedure demonstrated to be reliable to estimate soybean seed vigor, with comparable efficiency to discriminate seed lots similarly to recommended vigor tests.

Several researches have shown that infrared thermography can be widely applied in non-invasive examination of seed viability and vigor in different species (Kranner et al., 2010, Kim et al., 2013, Kim et al., 2014, Men et al., 2017, Liu et al., 2020). However, these authors used artificially aged seeds to study the efficiency of the method in assessment of seed vigor. This is the first time that infrared thermography is used to identify differences in vigor among original soybean seed lots, without artificial aging to achieve different levels of vigor.

This study demonstrated the viability of infrared thermography to detect thermal variations in the surface of soybean seeds and to associate it with vigor level of commercial seed lots. The proposed procedure was able to identify differences among seed lots in a similar way to other tests traditionally used to assess soybean seed vigor. The results showed that the measurement of the average temperature was an efficient parameter for ranking the seed lots in comparison to the maximum temperature, minimum temperature and thermal amplitude. In overall, the infrared thermography seems to be a quite promising procedure to identify differences in vigor among soybean seed lots both for populations and individual seeds, which may be very useful as a fast and non-invasive test in plant breeding research and in-house quality control programs established by seed companies.

4. Acknowledgments

The authors are grateful to M.Sc. Telma Paparotto Coelho (Animal Labor, Piracicaba, SP, Brazil) for the support in the thermographic measurements and for providing the laboratory facilities.

5. Funding

This work was supported by the São Paulo Research Foundation [grant number 2018/13139-0]; and the The Brazilian National Council for Scientific and Technological Development [grant number 402473/2016-7].

6. CRediT authorship contribution statement

Francisco Guilhien Gomes-Junior: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Visualization, Writing - original draft, Writing - review & editing. **Agide Gimenez Marassi:** Investigation, Methodology, Software, Visualization. **Lívia Araújo Rohr:** Visualization, Writing - review & editing. **Silvio Moure Cícero:** Visualization, Writing - review & editing.

7. Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

8. References

Baalbaki, R., Elias, S., Marcos-Filho, J., & McDonald, M. B. (2009). *Seed vigor testing handbook*. Ithaca: Association of Official Seed Analysts (Contribution, 32).

Baranowski, P., Mazurek, W., & Walczak, R. T. (2003). The use of thermography for presowing evaluation of seed germination capacity. *Proceedings of the International Conference on Quality Chains*, Vols. 1 and 2 – An Integrated View on Fruit and Vegetable Quality. Wageningen, Netherlands.

Brasil (2009). *Rules for Seed Testing.* (In Portuguese) Ministério da Agricultura, Pecuária e Abastecimento. Secretaria de Defesa Agropecuária. Brasília, DF: MAPA/ACS.

ElMasry, G., ElGamal, R., Mandour, N., Gou, P., Al-Rejaie, S., Belin, E., & Rousseau, D. (2020). Emerging thermal imaging techniques for seed quality evaluation: Principles and applications. *Food Research International, 131*, 1−16. https://doi.org/10.1016/j.foodres.2020.109025

Fessel, S. A., Panobianco, M., Souza, C. R., & Vieira, R. D. (2010). Electrical conductivity test of soybean seeds stored under different temperatures. (In Portuguese, with English abstract.) *Bragantia, 69*, 207−214. https://doi.org/10.1590/S0006-87052010000100026

Finch-Savage, W. E., & Bassel, G. W. (2016). Seed vigour and crop establishment: extending performance beyond adaptation. *Journal of Experimental Botany, 67*, 567−591. https://doi.org/10.1093/jxb/erv490

Hoffmaster, A. L., Fujimura, K., McDonald, M. B., & Bennett, M. A. (2003). An automated system for vigour testing three-day-old soybean seedlings. *Seed Science and Technology, 31*, 701−713. https://doi.org/10.15258/sst.2003.31.3.19

ISTA (2021). *International rules for seed testing*. Basserdorf, Switzerland: International Seed Testing Association.

Jianhua, Z., & McDonald, M. B. (1996). The salt accelerated aging test for small-seeded crops. *Seed Science and Technology, 25*, 123−131.

Kim, G., Kim, G. H., Ahn, C. K., Yoo, Y., & Cho, B. K. (2013). Mid-infrared lifetime imaging for viability evaluation of lettuce seeds based on time-dependent thermal decay characterization. *Sensors, 13*, 2986−2996. https://doi.org/10.3390/s130302986

Kim, G., Kim, G. H., Lohumi, S., Kang, J. S., & Cho, B. K. (2014). Viability estimation of pepper seeds using time-resolved photothermal signal characterization. *Infrared Physics and Technology, 67*, 214−221. https://doi.org/10.1016/j.infrared.2014.07.025

Kranner, I., Kastberger, G., Hartbauer, M., & Pritchard, H. W. (2010). Noninvasive diagnosis of seed viability using infrared thermography. *Proceedings of the National Academy of Sciences of the United States of America, 107*, 3912−3917. https://doi.org/10.1073/pnas.0914197107

Lahiri, B. B., Bagavathiappan S., Jayakumar T., & Philip J. (2012). Medical applications of infrared thermography: a review. *Infrared Physics and Technology, 55*, 221−235. https://doi.org/10.1016/j.infrared.2012.03.007

Liu, L., Wang, Z., Li, J., Zhang, X., & Wang, R. (2020). A non-invasive analysis of seed vigor by infrared thermography. *Plants, 9*, 1−12. https://doi.org/10.3390/plants9060768

Marcos-Filho, J. (2015). Seed vigor testing: an overview of the past, present and future perspective. *Scientia Agricola, 72*, 363−374. https://doi.org/10.1590/0103-9016-2015-0007

Marcos-Filho, J. (2016). *Seed physiology of cultivated plants* (2nd ed.). Londrina, PR: ABRATES.

Mastrodimos, N., Lentzou, D., Templalexis, Ch., Tsitsigiannis, D. I., & Xanthopoulos, G. (2019). Development of thermography methodology for early diagnosis of fungal infection in table grapes: The case of *Aspergillus carbonarius*. *Computers and Electronics in Agriculture,165*, 1−8. https://doi.org/10.1016/j.compag.2019.104972

Men, S., Yan, L., Liu, J., Qian, H., & Luo, Q. (2017). A classification method for seed viability assessment with infrared thermography. *Sensors, 17*, 1−14. https://doi.org/10.3390/s17040845

Prado, J. P., Krzyzanowski, F. C., Martins, C. C., & Vieira, R. D. (2019). Physiological potential of soybean seeds and its relationship to electrical conductivity. *Journal of Seed Science, 41*, 407−415. https://doi.org/10.1590/2317-1545v41n4214988

R Core Team (2019). *R Development Core Team*. A language and environment for statistical computing, *55*, 275−286.

Rodrigues, M., Gomes-Junior, F. G., & Marcos-Filho, J. (2020). Vigor-S: system for automated analysis of soybean seed vigor. *Journal of Seed Science, 42*, 1−12. https://doi.org/10.1590/2317-1545v42237490

Vieira, R. D., Bittencourt, S. R. M., & Panobianco, M. (2003). Seed vigour - an important component of seed quality in Brazil. *Seed Testing International, 126*, 21−22.

Xia, Y., Xu, Y., Li, J., Zhang, C., & Fan, S. (2019). Recent advances in emerging techniques for non-destructive detection of seed viability: A review. *Artificial Intelligence in Agriculture, 1*, 35−47. https://doi.org/10.1016/j.aiia.2019.05.001

List of figure captions

Figure 1. Schematic representation of the thermographic measurements and data analysis of soybean seeds: (1) seed imbibition in moistened paper towel rolls; (2) seed temperature homogenization by removing the seeds from the paper towel rolls and transferring them to a sheet of black EVA; (3) digital thermal images acquisition; (4) thermal image analysis and data correlation with other tests recommended for assessing the vigor of soybean seeds.

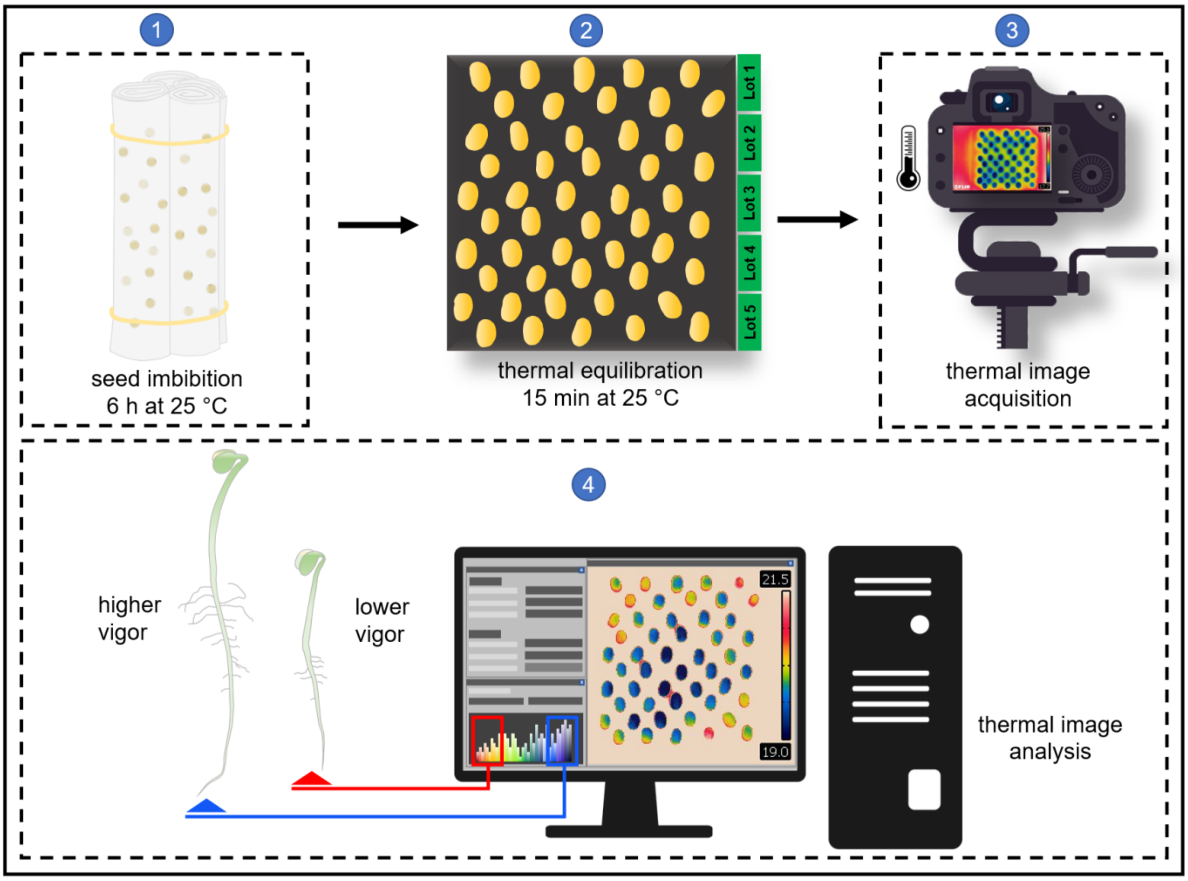


Figure 2. Results from maximum, minimum and average temperatures and thermal amplitude estimated in seeds of five lots of soybean. Extremities of each line represent the highest and lowest value and the symbols (●) are indicating the presence of outliers. In blue, numbers followed by lowercase letters correspond to mean comparisons (LSD test, $P \leq 0.05$).

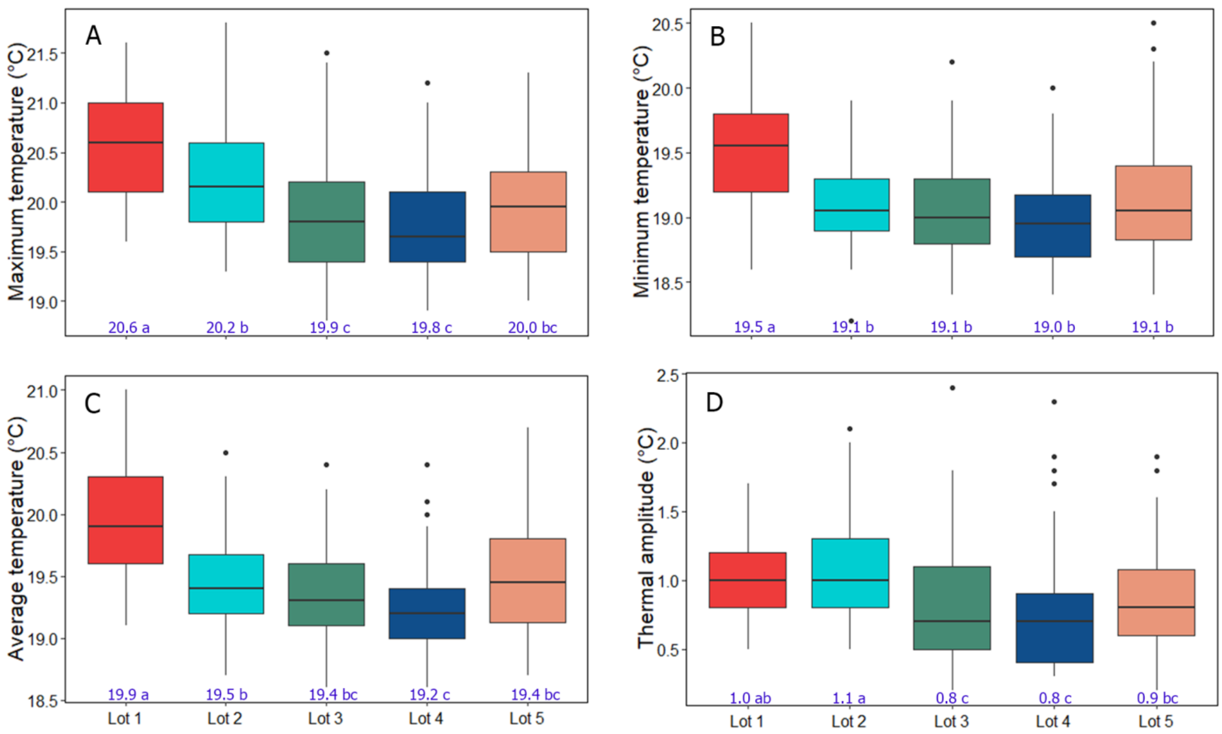
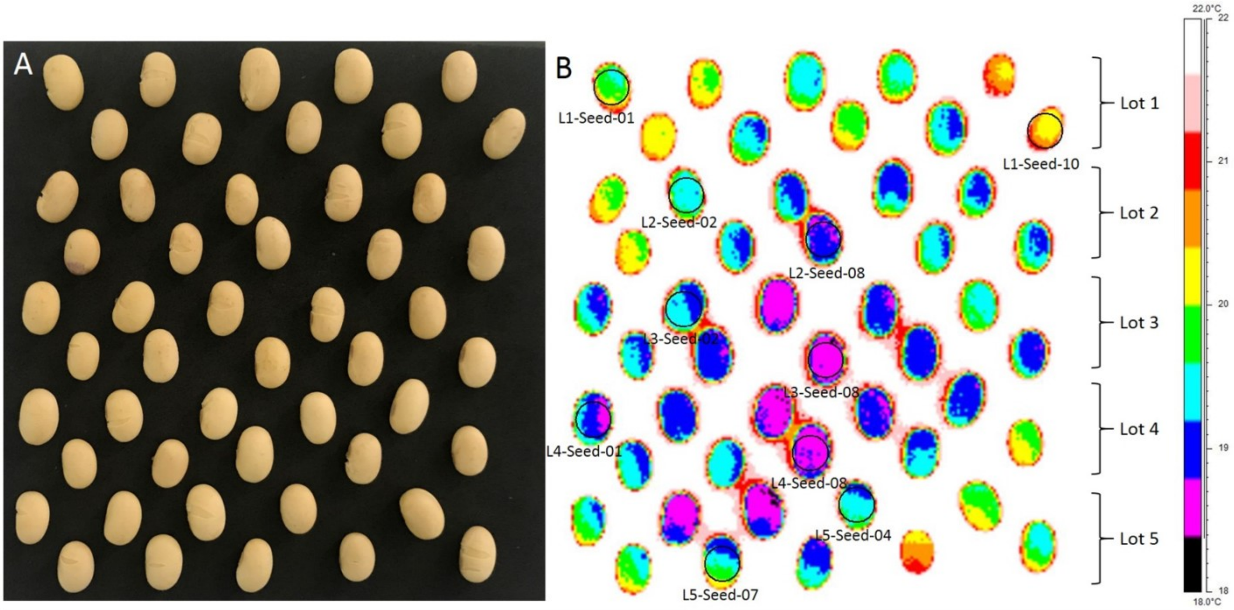


Figure 3. Color reflectance image (RGB) of 50 seeds representing the five seed lots (A) and respective thermographic images, highlighting two seeds from each seed lot (B). Area delimited by a circle corresponds to the ROI used to estimate the maximum temperature (Tmax), minimum temperature (Tmin), average temperature (Tavg) and the thermal amplitude (Tampl = Tmax − Tmin).



Temperature (°C)	L1-Seed-01	L1-Seed-10	L2-Seed-02	L2-Seed-08	L3-Seed-02	L3-Seed-08	L4-Seed-01	L4-Seed-08	L5-Seed-04	L5-Seed-07
Tmax	21.9	22.3	21.4	21.2	20.6	20.6	20.3	20.2	21.1	21.6
Tmin	19.4	20.0	19.1	18.7	18.9	18.4	18.5	18.6	19.0	18.9
Tavg	20.0	20.6	19.7	19.0	19.3	18.7	19.0	18.8	19.5	19.6
Tampl	2.5	2.3	2.3	2.5	1.7	2.3	1.8	1.6	2.1	2.7

Figure 4. Heatmap (A) showing the Pearson's correlation (p ≤ 0.05) among the data of physiological potential evaluations and seed surface temperature in soybean seeds. Biplot graphics (B) of principal component analysis among the relationship between germination (GER), first count of germination (FC), accelerated aging (AA), field seedling emergence (SE), electrical conductivity (EC), seedling length (SL), uniformity of seedling development (UNF), maximum temperature (Tmax), minimum temperature (Tmin), average temperature (Tavg) and thermal amplitude (Tampl). Labelled lines demonstrate the correlation of the data of physiological potential evaluations and seed surface temperature to principal component scores.

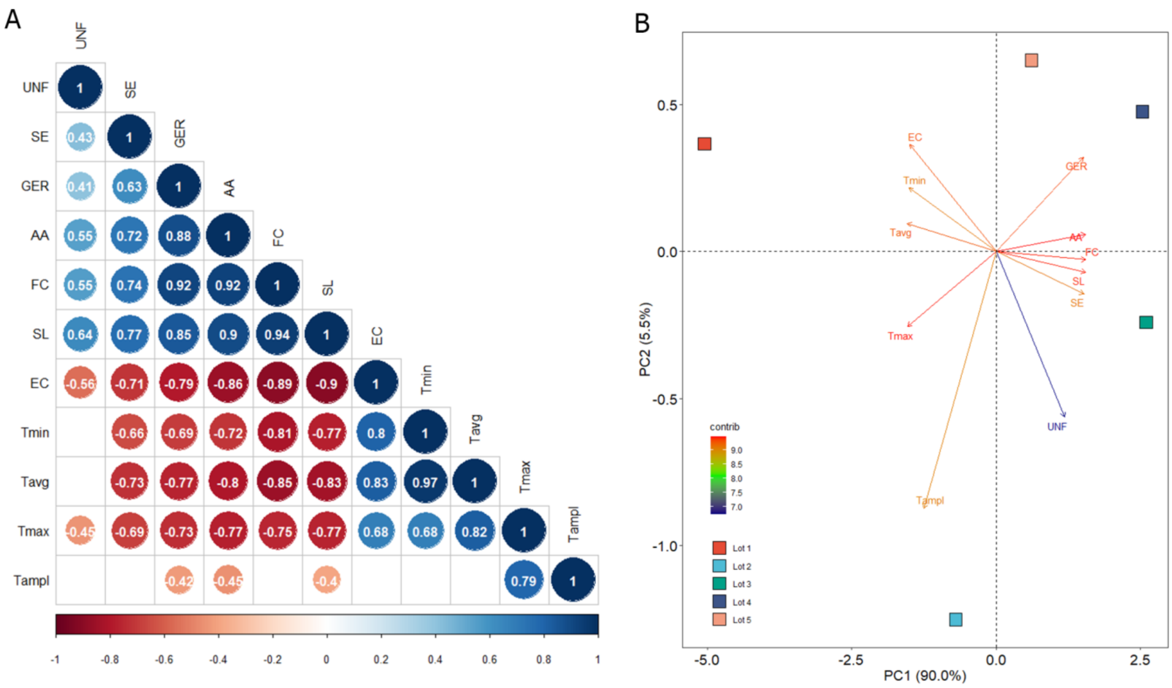


Table 1. Analysis of variance (ANOVA) table for the seed physiological evaluations.

Source of variation	Df	Sum of squares	Mean square	F value	*P*-value
			Germination (GER)		
Seed lot	4	1260.0	315.04	26.43	<0.0001
Error	20	238.4	11.92	-	-
Total	24	1498.6	-	-	-
			First count of germination (FC)		
Seed lot	4	6313.6	1578.4	101.7	<0.0001

Error	20	310.4	15.52	-	-
Total	24	6624.0	-	-	-
Accelerated aging (AA)					
Seed lot	4	4490.2	1122.56	48.89	<0.0001
Error	20	459.2	22.96	-	-
Total	24	4949.4	-	-	-
Field seedling emergence (SE)					
Seed lot	4	1439.04	359.76	8.72	0.0006
Block	4	216.64	54.16	1.31	0.30
Error	16	660.16	41.26	-	-
Total	24	2315.84	-	-	-
Electrical conductivity (EC)					
Seed lot	4	5693	1423.3	70.37	<0.0001
Error	20	404	20.2	-	-
Total	24	6097.6	-	-	-
Seedling length(SL)					
Seed lot	4	98.76	24.690	118.13	<0.0001
Error	20	4.18	0.209	-	-
Total	24	102.94	-	-	-
Uniformity of seedling development (UNF)					
Seed lot	4	107171	26792.9	7.73	0.0006
Error	20	69386	3469.3	-	-
Total	24	176557	-	-	-

Df = degrees of freedom.

Table 2. Physiological potential evaluations of five seed lots of soybean.

Seed lots	GER	FC	AA	SE	EC	SL	UNF
	%				$\mu S\ cm^{-1}\ g^{-1}$	cm	index
1	82 c[1]	52 d	52 d	59 b	118.5 c	2.1 c	629 c
2	87 b	78 c	71 c	74 a	85.4 b	5.4 b	709 b
3	98 a	96 a	89 a	79 a	75.9 a	7.4 a	816 a
4	98 a	93 a	88 a	80 a	80.4 ab	7.7 a	729 b
5	96 a	86 b	78 b	77 a	85.7 b	5.9 b	653 bc
LSD	2.03	2.32	2.82	3.78	2.65	0.27	34.75
CV (%)	3.76	4.88	6.33	8.69	5.04	8.02	8.33

Germination (GER), first count of germination (FC), accelerated aging (AA), field seedling emergence (SE), electrical conductivity (EC), seedling length (SL), uniformity of seedling development (UNF)

[1]Mean comparisons within each column (LSD test at an alpha level of 0.05).