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Equipment to assess vigor in soybean seeds using CO₂ produced during respiration¹

Equipamento para avaliar vigor em sementes de soja através do CO₂ produzido durante a respiração

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HIGHLIGHTS:

There is a need for new seed vigor tests based on the respiration process.

The equipment should be designed to reduce uncertainty in vigor tests.

A vigor test based on the CO₂ produced by the seeds' respiration is an excellent method to check the physiologic quality of seed lots.

ABSTRACT: The adoption of quick and reliable laboratory techniques and equipment to choose the best seed lots for marketing will influence the production of soybeans with superior physiological quality, among other areas in the sector. Therefore, the objective of this study was to evaluate the CO₂ concentrations produced by water-soaked soybean seeds and to verify the effectiveness of new equipment to help choose lots with different vigor levels. To evaluate the physical and physiological quality of the seeds, eight soybean lots were evaluated with the following tests: water content, weight of thousand seeds, first germination count, germination, electrical conductivity, emergence, and respiration evaluated by the Pettenkoffer apparatus and with equipment designed to measure CO₂ in seeds. The results were subjected to analysis of variance with means compared by Tukey's test at $p \leq 0.05$. Conventional methods showed significant differences in vigor and viability in soybean seeds. The equipment designed was efficient in detecting CO₂ produced by seeds soaked in water for 8 hours. The CO₂ readings with the equipment presented satisfactory results to predict the vigor in soybean seeds through respiration.

Key words: *Glycine max* L., MG811 sensor, quick tests, carbon dioxide

RESUMO: A adoção de técnicas e equipamentos laboratoriais rápidos e confiáveis para a escolha dos melhores lotes de sementes para comercialização influenciará a produção de soja com qualidade fisiológica superior, entre outras áreas do setor. Diante disto, objetivou-se avaliar as concentrações de CO₂ produzido por sementes de soja embebidas em água e comprovar a eficácia de um novo equipamento que auxilie na seleção de lotes com diferentes níveis de vigor. Para avaliação da qualidade física e fisiológica das sementes, oito lotes de soja foram avaliados com os seguintes testes: teor de água, peso de mil sementes, primeira contagem de germinação, germinação, condutividade elétrica, emergência e respiração avaliadas pelo aparelho Pettenkoffer e com equipamento projetado para medir CO₂ em sementes. Os resultados foram submetidos à análise de variância com médias comparadas pelo teste de Tukey a $p \leq 0,05$. Os métodos convencionais evidenciaram diferenças significativas do vigor e viabilidade em sementes de soja. O equipamento desenvolvido se mostrou eficiente na detecção de CO₂ produzido pelas sementes embebidas em água durante 8 horas. As leituras de CO₂ com o equipamento apresentaram resultados satisfatórios para predizer o vigor em sementes de soja por meio da respiração.

Palavras-chave: *Glycine max* L., MG811 sensor, testes rápidos, dióxido de carbono

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INTRODUCTION

Seed vigor is the set of properties that determines the physiological potential for rapid and uniform emergence and development of normal seedlings under a wide range of environmental conditions (Rocha et al., 2017). The ambient temperature, water content, and chemical composition of the seeds influence the preservation of physiological quality by altering biological activities and accelerating the respiratory one (Tillmann et al., 2019).

During this process, the stored glucose is used as an energy source, consuming oxygen, and producing CO₂ with subsequent release of water and energy (Silva et al., 2017). The released CO₂ comes from the complete oxidation of carbohydrates and lipids, which are consumed during the degradation of these reserves in the germination process (Marcos Filho, 2015; Wendt et al., 2017).

Depending on the temperature, humidity, and gas concentration to which the seeds are exposed, these metabolic reactions can vary significantly. Therefore, researchers have opted for reliable and economically viable checks, which help in deciding the fate of certain seed lots through vigor tests to identify less advanced stages of deterioration, according to Wendt et al. (2017).

Promising results were obtained by Aumonde et al. (2012) in kidney bean seeds, and by Mendes et al. (2009) and Dode et al. (2013) in soybean seeds using the Pettenkoffer apparatus. However, Oliveira et al. (2015), while evaluating the respiratory activity in watermelon seeds, observed that there was no positive and significant correlation between respiration and other vigor tests, corroborating with Dranski et al. (2019).

In this context, the objective of this study was to evaluate the CO₂ concentrations produced by soybean seeds soaked in water and to prove the effectiveness of new equipment that helps in choosing lots with different levels of vigor.

MATERIAL AND METHODS

The experiment was performed from January to June 2019, in the Laboratory of Seed Physiology, and the Laboratory of Agrotechnology, at the Federal University of Pelotas. Eight soybean seed lots benefited and had statistically similar germination and greater than 80 percent were used, according to IN n° 45 of MAPA (Brasil, 2013). Owing to the low initial moisture (9.5 ± 0.5%), seeds were preconditioned until 11.8 ± 1.2% moisture (w.b.).

For this, they were evenly distributed on braided wire screens and placed in Gerbox[®] boxes containing 40 mL of distilled water under ambient conditions (25 ± 5 °C). The duration for which the seeds were in the boxes was based on preliminary tests (18 to 24 hours). After preconditioning, physical and physiological quality analyses were performed using the following tests:

Weight of a thousand seeds (PMS) was evaluated by weighing on analytical scale (0.1 mg) of 8 sub-samples of 100 seeds, from the portion of pure seeds in each lot (Brasil, 2009). The results were expressed in grams.

In conducting the germination test, five replicates of 50 seeds were used, distributed over two sheets of Germitest[®] paper, moistened with 2.5 times the dry weight of the paper, packed with Biochemical Oxygen Demand (BOD) type germinators at 25 °C with a 12-hour photoperiod, whose counts were performed on the 5th and 8th days, according to Brasil (2009). The results are expressed as percentages.

First germination count (PCG): obtained from the quantification of the number of seedlings accumulated on the first counting day, performed on the fifth day of the germination test. The results are expressed as percentages.

Electrical conductivity test (EC): 250 seeds were used, distributed in five replicates of 50 seeds, weighed, and placed in plastic cups containing 75 mL of deionized water for 24 hours in an air-conditioned room (25 °C). Then, the quality of seeds was evaluated, with the aid of a conductivity meter (Carvalho et al., 2009). The results are expressed in μS cm⁻¹ g⁻¹ of seed according to Eq. 1.

$$CE = \frac{\text{reading solution with seeds} - \text{reading water}}{\text{mass dry seeds}} \quad (1)$$

Accelerated aging (EA) was conducted with five replicates of 50 seeds, arranged in a single layer on a steel screen inserted into plastic boxes (Gerbox) containing 40 mL of distilled water (Krzyzanowski, 1991). Subsequently, the boxes were transferred to a germination chamber type BOD at 41 °C for 48 hours. The seeds were then subjected to the germination test, as previously described. The evaluation was carried out on the fifth day after sowing, and the seedlings were considered normal, according to Brasil (2009). The results are expressed as the percentage of normal seedlings.

Emergence (E%): 5 replicates of 50 seeds from each lot were used. The evaluation was based on the final count of the total seedlings that emerged for each lot, carried out 16 days after sowing when the percentage of seedlings remained constant. The results were expressed as percentages. Soil moisture was maintained close to field capacity through daily irrigation with the aid of a watering can.

The assessment of the CO₂ produced by the seeds during respiration was performed using equipment that uses MG811 sensor, and with the aid of the Pettenkoffer device, according to Mendes et al. (2009), using 5 replicates of 10 g of seeds, weighed on an analytical balance (0.1 mg). Respiratory activity was calculated based on the equation proposed by Müller (1964).

The equipment subject to patent (BR 10 2019 023534 9) transmits the data collected by the MG811 sensor and through electronic boards and circuits, expresses the values on an LCD display (16 x 2, 16-pin). To capture CO₂, the sensor remained tightly coupled to a glass container (200 mL) with the seeds inside.

Under laboratory conditions (25 ± 2 °C) for 8 days, the data were obtained every 30 min, for 8 hours, using three replicates of 100 seeds immersed in a volume of 25 mL of distilled water in an airtight container containing the coupled sensor. The period and volume of water were established based on preliminary tests. The values were expressed in millivolts (mV). The analysis

of the breathing data mentioned here was performed using descriptive statistics (mean, minimum, median, maximum, and standard deviation).

With the aid of the statistical program Rbio 1.0, the results were tested for normality (Shapiro-Wilk) and homoscedasticity (Brown-Forsythe) and, if complied with ANOVA assumptions, they were subjected to analysis of variance with means compared by the Tukey test at $p \leq 0.05$.

Data on the mass of a thousand seeds, first germination count, accelerated aging, and respiration using the Pettenkoffer method showed normal distribution, eliminating the need for data transformation. However, the water content, germination, and emergence variables were transformed to $\arcsin \sqrt{(x/100)}$ and, for $\log(x)$ to the electrical conductivity variable.

RESULTS AND DISCUSSION

The results for the water content variable show differences between the tested lots, classifying lots 3, 4, 5, and 7, with means statistically similar and superior to the others (Table 1). According to Tunes et al. (2012), when the water content of the seeds is relatively low, greater reliability is allowed for the results obtained in the physiological quality tests, because, within certain limits, the more humid seeds are, the more susceptible they are to deterioration.

However, according to Barbosa et al. (2012) and Paixão et al. (2017), the water content of the seeds directly influences the integrity of the membranes, as assessed by the electrical conductivity test, and can influence other analyses of physiological quality.

The evaluations of the first germination count referred to by Martins et al. (2017) as an indication of vigor, showed significant differences between lots (Table 1), with the lowest means observed in lots 1 and 8. Eventually, this variable better expresses the differences in germination speed between seed lots (Martins et al., 2017), which makes it liable to fail, indicating the performance of a population in totally favorable conditions, and to benefit many lots of medium vigor.

The germination evaluation (Table 1) allowed the classification of the lots at three levels of viability (high, medium, and low). Despite the statistical differences observed, all lots showed germination higher than the minimum recommended for commercialization (80%), according to IN

nº 45, September 17, 2013 (Brasil, 2013), which is preferable for physiological quality analyses.

The chemical instability of lipids is one of the main factors in reducing the germination speed of several species (José et al., 2010); therefore, lipid peroxidation and oxidative stress cause the deterioration of seeds during their aging.

Through the accelerated aging test (Table 1), it was possible to classify lots 4 and 5 as high vigor (71% and 68%, respectively) and suggest that the other lots showed low vigor, with low tolerance to adverse conditions, since the results of the emergence in the sowing bed were superior to those of accelerated aging, showing little or no stress conditions during the emergence.

Greater water absorption during accelerated aging can promote further deterioration and, consequently, prevent the seed lot from expressing its maximum physiological potential (Tunes et al., 2012). However, the use of saline solutions promotes the reduction of relative humidity and water absorption by the seeds, thereby reducing the deterioration and favoring the expression of the vigor of the lot (Tunes et al., 2012).

Cellular damage assessed by the electrical conductivity of seeds in lots 1, 3, and 8 (Table 1) allowed them to be classified as having low vigor, as well as in EA and EC. Injuries caused by beneficiation or even during imbibition may have caused irreversible damage, causing the rupture of membranes and the release of exudates, showing more severe effects in lots with lower water content (Barbosa et al., 2012; Gordin et al., 2015; Paixão et al., 2017).

The differences between the lots when evaluating the weight of a thousand seeds (Table 1) are not associated with vigor, considering that some lots with higher PMS (Lots 1, 3, 4, 5, and 8) presented medium or low vigor. As reported by Conrad et al. (2017), the weight of a thousand seeds is influenced by the size of the sieve in which they are classified during beneficiation.

Hampton et al. (2013) also stated that there is no relationship between seed mass and vigor and germination in seed lots. However, the C/N ratio may result in a reduction in the capacity of seeds to meet the requirement of amino acids for the synthesis of proteins necessary for embryo growth and seed germination.

According to Table 1, seed respiration evaluations by the Pettenkoffer apparatus were not efficient in classifying

Table 1. Mean values for the variables water content (TA), germination (G), electrical conductivity (EC), and field emergence (E), first germination count (PCG%), accelerated aging (EA%), respiration by the Pettenkoffer, and weight of a thousand seeds (PMS) in eight lots of soybean seeds

	Transformed variables			E	Variables without transformation			
	TA	G	EC		PCG	EA	PMS	Pettenkoffer
	(%)	(%)	($\mu\text{S cm}^{-1} \text{g}^{-1}$)	(%)	(%)	(g)	($\text{mg CO}_2 \text{g seed}^{-1} \text{min}^{-1}$)	
Lot 1	0.33 (10.41) c	1.18 (85) c	2.87 (746.2) a	1.11 (80) c	67 b	50 cd	226.29 a	0.0037 a
Lot 2	0.33 (10.27) c	1.35 (95) ab	2.65 (444.8) f	1.30 (93) ab	90 a	58 bc	146.67 d	0.0038 a
Lot 3	0.35 (11.79) a	1.39 (96) ab	2.79 (625.0) b	1.26 (90) bc	91 a	50 cd	190.99 b	0.0035 a
Lot 4	0.35 (12.05) a	1.35 (94) ab	2.75 (566.7) cd	1.45 (98) a	90 a	71 a	190.94 b	0.0033 a
Lot 5	0.35 (11.49) ab	1.43 (98) a	2.72 (523.8) de	1.34 (95) ab	95 a	68 ab	192.76 b	0.0042 a
Lot 6	0.33 (10.53) bc	1.42 (97) a	2.67 (463.5) f	1.26 (90) bc	90 a	43 d	152.40 d	0.0036 a
Lot 7	0.35 (11.60) a	1.26 (91) bc	2.71 (512.1) e	1.24 (89) bc	86 a	51 cd	175.49 c	0.0035 a
Lot 8	0.31 (10.6) d	1.19 (86) c	2.76 (579.5) bc	1.20 (87) bc	72 b	47 cd	189.40 b	0.0043 a
CV (%)	4.60	3.07	4.31	4.97	5.78	11.30	2.03	16.25

Mean values for TA, G, EC and E were transformed into $\arcsin \sqrt{(x/100)}$, $\arcsin \sqrt{(x/100)}$, $\log(x)$ and $\arcsin \sqrt{(x/100)}$, respectively; Means followed by the same letter in the column do not differ statistically from each other by Tukey's test at $p \leq 0.05$; CV - Coefficient of variation

the lots at different levels of vigor, since the observed values did not differ statistically by the Tukey test ($p > 0.05$). These results do not corroborate those obtained by Mendes et al. (2009), Aumonde et al. (2012), and Leite et al. (2018), when assessing respiration in soybean, kidney beans, and okra seeds, respectively.

According to Lamarca & Barbedo (2012), the water content and temperature influence the respiratory metabolism of the seeds, so that the increase in O_2 consumption without the corresponding release of CO_2 suggests the inefficiency of antioxidant systems in the seed. Besides, not all carbon in the respiratory route will become CO_2 , with intermediates that will be used in the synthesis of amino acids, lipids, and other compounds (Chen & Arora, 2011).

The difference observed between the consumption of O_2 and the production of CO_2 by the seeds, according to Lamarca & Barbedo (2012), may also be related to the use of fatty acids as an initial substrate for respiration, producing less CO_2 per mole of oxygen. During the incubation of *Caesalpinia echinata* seeds, Lamarca & Barbedo (2012) measured the levels of O_2 consumed and CO_2 produced and found that seeds with a water content of 37% produced $60 \mu\text{mol g}^{-1}$ of dry mass day^{-1} , and that seeds with 6% water content and O_2 consumption were three times greater than CO_2 release.

Regarding seed respiration evaluated with the equipment subject to the patent (Figure 1), in the first 2 hours there was no relevance in the data owing to the volume of water in the bottle and the need for gas stabilization, which would eliminate the interference of CO_2 from the environment. In addition, an initial situation of hypoxia could occur in the early stages of seed imbibition (Marinho et al., 2019).

Thus, according to Figure 1, the respiration of lots 1 and 4 remained stable during the evaluations, although lot 1 had higher values, proving the hypothesis that less vigorous lots have greater respiration and, consequently, greater deterioration (Tillmann et al., 2019), as proven by the emergence and accelerated aging, as shown in Table 2. This was also observed for lots 2 and 3, whose increase in respiration in lot 2 was noticed from two and a half hours to the five-hour

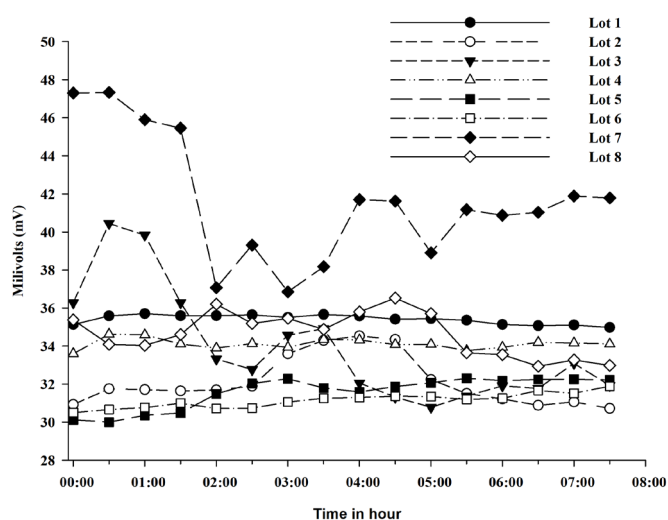


Figure 1. Respiration in soybean seeds in function of time after moistening by means of readings using the equipment subject to patent

evaluation period. During this period, enzymatic activation, and metabolites necessary for germination occur, characterized by phase 1 of imbibition (Carvalho et al., 2012).

According to Carvalho et al. (2012), soybean seeds present radicle protrusion 38 hours after installing the germination test, a fact confirmed by the imbibition curve presented by the authors. The germination phases presented in that work were: phase 1 until 10 hours; phase 2 until 25 hours; and phase 3 until 38 hours.

According to Tillmann et al. (2019), the expression of vigor in seeds under less favorable conditions can be better quantified owing to the degradation of reserves and the consequent release of CO_2 . Therefore, in high vigor seeds, the CO_2 levels will be lower than those observed in low-quality lots. However, there is disagreement regarding the association of vigor and the concentration of CO_2 produced by the seeds (Dranski et al., 2019).

The values obtained for seed lot 5 show a slight increase in breathing after the first hour of evaluation, whose values indicate levels of vigor lower than in lot 6. However, lot 7 expressed oscillation and high values in the first 3 hours of evaluation (Figure 1), which was also confirmed by the values presented in Table 2.

The increase in respiration after 3 hours of evaluation is a consequence of cellular reorganization and a lower volume of water after imbibition and exposure to O_2 in the container. According to Metivier & Paulilo (1980) and Marcos Filho (2015), the respiratory rate in beans and soybean seeds, respectively, is higher in the first 6 hours of imbibition.

In the respiration method used by Metivier & Paulilo (1980), bean seeds were subjected to 12 hours of soaking in distilled water and used a Warburg respirometer at 30°C , with sucrose and KOH solution, as well as filter paper and thermometer. However, the time the containers remained open may have influenced the CO_2 levels fixed on the filter paper strip.

Concerning seed lot 8, in the period from 1 hour 30 min to 5 h 30 min of evaluation, there was an increase in CO_2 levels because of imbibition, consumption of O_2 , and beginning of the germination process (Carvalho et al., 2012). This seed lot had medium vigor levels, possibly owing to the low water content, which compromised the evaluation of PCG and germination (Table 1).

According to Lamarca & Barbedo (2012), the physiological processes involved in the loss of viability of some seeds may

Table 2. Means and complementary descriptive statistics of respiration data evaluated for 8 hours by the equipment

Lot	Mean (mV)	Minimum	Median	Maximum	Standard deviation
1	362.50 b	356.70	362.10	370.80	3.75
2	329.00 d	307.10	322.30	408.90	22.74
3	347.30 c	311.10	334.70	462.90	35.81
4	349.30 c	319.70	349.20	364.70	9.64
5	323.40 d	302.90	325.50	347.30	15.36
6	318.90 d	305.90	318.30	335.90	7.34
7	426.50 a	336.10	426.60	505.60	30.32
8	354.70 bc	323.20	353.30	397.70	17.68

Means followed by the same letter in the column do not differ statistically from each other by Tukey's test at $p \leq 0.05$

be related to seed respiration or oxidative reactions, such as lipid peroxidation, significantly influencing the reorganization of membranes and resumption of the germination process, resulting in low vigor seeds.

Seeds with advanced levels of deterioration tend to have greater cellular damage, among which the activity of mitochondria can also be compromised, resulting in reduced breathing and generation of adenosine triphosphate (ATP) (Taiz et al., 2017). However, it is likely that during the process of cellular reorganization, the greater metabolic and respiratory activity will occur, according to Dode et al. (2013) and Venske et al. (2014).

Therefore, when comparing the results between the two methods to assess respiration and vigor in soybean seeds, the new equipment using a CO₂ sensor demonstrated differences between the lots evaluated at all time intervals (Figure 1 and Table 2), whereas, by the Pettenkoffer apparatus, the evaluations did not result in a significant effect.

CONCLUSIONS

1. Conventional methods showed significant differences in vigor in different lots of soybean seeds.

2. The equipment subject to the patent proved efficient in the detection of CO₂ produced by soybean seeds soaked in water for eight hours, allowing the estimation of the vigor in soybean seeds through respiration.

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