

Carbon dioxide quantified by the infrared in evaluation of respiratory activity of wheat seeds¹

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ABSTRACT

The objective of this study was to develop and validate the methodology of carbon dioxide concentration in the evaluation of vigor of *Triticum aestivum* L. seeds based on infrared spectroscopy. The proposed method quantifies CO₂ content using a drag system and exhaustion to the gas released by the seeds. Samples of 50 seeds of six lots of cultivar CD 123 were incubated at temperatures of 15, 25 and 40 °C. The CO₂ content released after 1, 3, 6, 9, 12 and 24 h of incubation were quantified. Additionally, the percentage of normal seedlings emerged in the field were evaluated. The simple correlation coefficients were evaluated among tests. After determining the reading conditions, the precision and accuracy of the proposed method were evaluated, using 15 lots of seeds. For the evaluation of the respiratory activity in *Triticum aestivum* seeds, a sample of 25 seeds, incubated at 25 °C for a minimum of 12 h is recommended since it allows to classify lots with different levels of vigor and predict the establishment of seedlings in the field, being the appropriate method for measuring CO₂ as it externalizes precision between successive measurements and agreement with the reference method.

Key words: CO₂; infrared spectroscopy; *Triticum aestivum* L.; validation method.

RESUMO

Dióxido de carbono quantificado pelo infravermelho na avaliação da atividade respiratória em sementes de trigo

Objetivou-se desenvolver e validar a metodologia da concentração de dióxido de carbono na avaliação do vigor de sementes de *Triticum aestivum* L. com base na espectroscopia no infravermelho. O método proposto quantifica o conteúdo de CO₂ através de um sistema de arraste e exaustão do gás liberado pelas sementes. Amostras de 50 sementes, de seis lotes da cultivar CD 123 foram incubadas sob temperaturas de 15, 25 e 40 °C. Quantificou-se o conteúdo de CO₂ liberado após 1, 3, 6, 9, 12 e 24 h de incubação. Paralelamente, avaliou-se a porcentagem de plântulas normais emergidas em campo. Foram calculados os coeficientes de correlação simples entre os testes. Após a determinação das condições de leitura, avaliou-se a precisão e exatidão utilizando 15 lotes de sementes. Para a avaliação da atividade respiratória em sementes de *Triticum aestivum* recomenda-se que amostras sejam incubadas a 25 °C por um período mínimo de 12 h, o que possibilita classificar lotes em diferentes níveis de vigor e prever o estabelecimento de plântulas em campo, sendo o método adequado para a mensuração de CO₂, pois externa precisão entre medidas sucessivas e concordância ao método de referência.

Palavras-chave: *Triticum aestivum* L.; CO₂; espectroscopia no infravermelho; validação.

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INTRODUCTION

Wheat (*Triticum aestivum* L.) is a cereal of great importance in the world for human and animal nutrition. It is considered a dominant crop in areas of subtropical and temperate climate; it has a significant impact on the world agricultural economy. In Brazil, whose domestic consumption is estimated at 12.25 million tons, a production of 7.89 million tons is projected for 2019, so the Brazilian production of this cereal is still lower than the supply demand (Mapa, 2016).

The investigation for self-sufficiency in production and higher productivity partly depends on the quality of the seeds used in the crop. Vigor tests have been used in tools used by the seed industry to eliminate low-quality seed lots for commercialization. Respiratory activity is one of the first biological manifestations of loss of vigor (Rosental *et al.*, 2014) and its detection may help in the monitoring and control of the quality of seed production by the seed industries.

Respiratory activity is measured through the consumption of oxygen, calorimetry, and carbon dioxide (CO₂) content released by the respiratory process via titration, fluorescence, and gas chromatography (Mendes *et al.*, 2009; Schabes & Sigstad, 2011). However, these methods require time, skilled labor and present high operational costs, limiting their diffusion in seed analysis and laboratory routines.

Carbon dioxide can also be quantified by infrared spectroscopy (Dranski *et al.*, 2013; Dantas *et al.*, 2015). This technology enables the analysis of non-destructive samples and repeated measurements of a single seed or even a seed sample either in the dry state or with germination in progress in a short time, but little information on its use in evaluating seed vigor is available.

Although these studies already present indicatives of a methodology for the evaluation of the CO₂ released by the seeds, the alternative method proposed by the present work suggests the modification of the collection and expression of the results obtained by a gas analyzer. Consequently, the indication of a certain test must be preceded by methodological validation procedures (Kataoka *et al.*, 2011).

The evaluation of the CO₂ concentration by the proposed infrared consists of exhausting the contents of the gas released by the seeds, kept in a closed system, up to the gas meter detection chamber. The meter chamber remains at constant CO₂ flow and concentration, serving as the baseline, much like gas chromatography. When content dragging occurs, a peak similar to that of a chromatogram can be detected and constructed, whose area represents its content, therefore, capable of being compared.

Several factors may influence the result, making it necessary to define a methodology that allows the separation of seed lots more accurately, since the incubation temperature and the time necessary for the evaluation can affect the respiratory rates, in the case of seeds with high physiological potential.

The objective of this study was to develop and validate the methodology of carbon dioxide concentration in the evaluation of vigor of *Triticum aestivum* L. seeds.

MATERIAL AND METHODS

Development of the carbon dioxide concentration method

Seed sample and physiological quality characterization

Six basic seed lots of *Triticum aestivum* of cultivar CD 123 were used, granted by Cooperativa Central de Pesquisa Agrícola – COODETEC (Central Cooperative of Agricultural Research). The seeds were from the 2013 crop, with moisture content of $14.1 \pm 0.3\%$, and 30.8 ± 1.2 g of the average weight of one thousand seeds. The seeds were received with no treatment with pesticides. During the experiment, the seeds were stored in Kraft paper boxes and kept in a dry chamber adjusted to 20 ± 2 °C, with a relative humidity of $40 \pm 3\%$.

The following were determined: **Germination** - The germination test was conducted with four replicates of 100 seeds, using the germitest paper roll method, packed in a germinator at 20.0 ± 2.0 °C, in a 12-h photoperiod. The results were expressed as a percentage of normal seedlings obtained on the eighth day after sowing, as recommended by the Rules for Seed Analysis (Brasil, 2009). **First Germination Count** - The test was carried out simultaneously to the germination test by counting the percentage of normal seedlings obtained on the fourth day after sowing (Brasil, 2009). **Seedling dry mass** - It was obtained from the normal seedlings resulting from the germination test, removed after the second count, dried in an air circulation oven at 80 °C for 24 h and the results expressed in mg per seedling (ISTA, 1995). **Potassium leaching** - The test was conducted with four replicates of 50 seeds, packed in containers containing 75 mL of deionized water at 25 °C for three hours. The readings were obtained with the aid of a flame photometer and the results expressed in $\mu\text{mol g}^{-1}$ of seeds (Favarato *et al.*, 2011). **Electrical conductivity** - The test was conducted with four replicates of 50 seeds, packed in containers containing 75 mL of deionized water at 25 °C for 18 h (Loeffler *et al.*, 1988). **Field emergence** - The test was conducted with four blocks of 100 seeds that were sown in August 2013, in furrows with 5.0 m in length and 3.0 cm in depth, with furrows spaced by 30 cm, under conditions of the field, without irrigation. The percentage of emerged

normal seedlings was computed on day twenty-one after sowing (Nakagawa, 1994).

Determination of carbon dioxide concentration

Quantification of CO₂ was performed by assessing the CO₂ content released during respiration with the aid of an IRGA gas exchange meter (LI-COR 6400 XT). An injection system and a CO₂-free air flow, coupled with the equipment, were fitted with an airflow compressor set in 140 mL min⁻¹, which injects air into a 50 mL-jar filled with soda lime properly sealed and with a rubber septum for injectables. Plastic hoses with a diameter of 3.0 mm and a length of 15 cm were used for connecting the system formed by the compressor, soda lime container, and sample jar, and disposable hypodermic needles (1.6 mm x 40 mm) were coupled at the ends. A segment of plastic hose with the same characteristics described above was attached to the equipment, one end being fixed in the IRGA gas chamber and the other inserted in the sample bottle. Therefore, two needles were inserted into the sample flask at reading, and then the air flow system was run simultaneously with the equipment.

For sample preparation, four replicates of 50 seeds were used, accommodated in 50-mL glass containers, and then added with deionized water, whose volume allowed to reach a moisture content of 30% (m/m), according to Equation 1, then closed with a rubber septum for injectables and properly sealed. The containers were then incubated in BOD-type chambers at temperatures of 15, 25 and 40 °C.

$$\text{Vol (g)} = \left[\text{MA} - \frac{\text{MA} - \left[\text{MA} * \frac{\text{GU}}{100} \right]}{1 - (0.01 * \text{GUD})} \right] \quad (1)$$

Where: MA – sample mass (g); GU: moisture content (%); GUD – moisture content to be reached (%).

Readings for carbon dioxide concentration were performed at 1, 3, 6, 9, 12 and 24 h of incubation. Regarding achievement of the value of each repetition, the equipment was self-programmed to capture the CO₂ concentration every 0.5 s until the complete exhaustion of the contents. The gas analyzer chamber was maintained at a constant concentration of 380 µmol of CO₂, with a flow of 500 µmol s⁻¹. From the values of each repetition, the gas peaks were adjusted to a log-normal distribution and the peak area was obtained, according to Equation 2 proposed by Felinger (1998) and the results expressed in mmol of CO₂ per gram of seeds.

$$f(t) = \frac{A}{X} \text{EXP} \left[-0.5 \left(\frac{\ln(t - t_0)}{\sigma} \right)^2 \right] \quad (2)$$

Where: A = peak area (µmol CO₂);

Data analysis

A completely randomized design was used for the evaluation of the physiological quality. The emergence in the field used the randomized block design. In the CO₂ concentration evaluation, a randomized block design was used in a factorial arrangement (6 x 6), comprising six lots and six incubation time periods. The incubation temperature factor was approached alone.

The data obtained were investigated as to the normality of residue distribution by the Shapiro-Wilk test, and the homogeneity of the variance by the Bartlett test. Subsequently, the data were submitted to analysis of variance. When there were statistically significant differences, the means between the lots were grouped by the Scott-Knott test at 5% probability of error. For the period, the regression analysis was used. Then, the simple correlation coefficients between the carbon dioxide concentration and the results of the vigor and field performance tests were obtained at the level of 5% of probability of error by the t test.

Carbon dioxide concentration method validation

Fifteen lots of basic seeds of *Triticum aestivum* cultivar CD 123, harvested in 2013, with a moisture content of 10.2 ± 0.2% were used. The percentage of germination as described above was evaluated.

The concentration of CO₂ was obtained in 20 replicates of 50 seeds that were placed in 50-mL glass containers, then added with deionized water, whose volume allowed to reach the humidity of 30%, according to Equation 1. The containers were incubated in BOD type chambers at temperatures of 25 °C for 12 h. Expression of the results occurred as described previously. After obtaining the results, the Grubbs test was applied to the level of 95% of error probability for detection of extreme values.

For the validation of the developed method, the validation parameters of the precision and accuracy proposed by ISO 5725-2 (1994) were used. **Precision** – Precision analysis was performed by repeatability analysis by comparing critical repeatability limit (CRL) with total amplitude (Å) at the 1% error probability level and by analysis of variance. For this, ten samples were read on two different days for the same lot. For the comparison of the days of analysis by lot, the analysis of variance was used at the level of 5% of error probability. **Accuracy** – Accuracy analysis was performed by comparing methods using the gas chromatography results as the reference. For that purpose, 2-mL samples were extracted from four replicates per lot with the aid of a 2.5-mL analytical syringe and then injected into a gas chromatograph equipped with a methanator, flame ionization detector, and hydrocarbon capillary column.

Concentration of CO₂ was quantified by comparing the areas of the chromatographic peaks of the samples and the analytical standard of CO₂; the results were expressed in mmol CO₂ g⁻¹. The simple correlation coefficient between the results obtained by infrared and gas chromatography (reference) was calculated, and the t test was applied at a 5% probability of error in the comparison of the methods.

RESULTS AND DISCUSSION

Determination of carbon dioxide concentration

The evaluation of the physiological quality indicated the occurrence of significant differences in the percentage of normal seedlings of the germination test (Table 1). Two groups were formed, where the one with the lowest viability resulted in a germination of 94% and the other lots with a mean of 98%, with all lots showing a germination percentage greater than the minimum (70%) recommended by Normative Instruction 45/2013 of the Ministry of Agriculture, Livestock and Supply, which deals with the establishment of identity and quality standards for the production and commercialization of *Triticum aestivum* seeds (Brasil, 2013).

The vigor assessment indicated that membrane integrity measured by potassium leaching tests and electrical conductivity (Table 1) were the most sensitive tests in the separation of lots with similar germination, due to the formation of three groups. Such groups covered lots 2 to lot 6, suggesting that the highest velocity of restoring of cell membrane integrity during imbibition results in lower releasement of solutes to the outer media in higher vigor seeds (Marcos Filho, 2015). The loss in the integrity of biological membranes is an effect caused by the deterioration that occurs in advance to the loss of germination speed and seedling growth (Benamar *et al.*, 2003) because these physiological effects evaluated by the test of the first germination count and dry mass of seedlings, respectively, only allowed the formation of two groups of the respective plots.

When the results of laboratory tests are compared with field performance (Table 1), it was observed that tests based on membrane integrity allowed to predict the field performance of wheat seeds, considering that the lots with the highest percentage of emergence in the field (lots 5 and 6) differed between exudate release rates, while the other tests did not present detection of sensitivity.

The loss of vigor is a result of physiological, biochemical, physical and ontogenetic changes that occur in advance of the loss of viability (Menezes *et al.*, 2014), which corroborates with the results of the vigor tests for intermediate emergency rate lots (Lots 2, 3 and 4).

The concentration of CO₂ released by wheat seeds was adjusted to the exponential model when incubation was carried out at temperatures of 15 and 25°C (Figure 1). For samples incubated at 40°C, the data were adjusted to a sigmoidal model of three parameters, and after 18 h, the point of maximum inflection of the curve was obtained, indicating that from this moment, the increase in CO₂ concentration is minimum and stability begins, culminating in the threshold. This moment is indicative that even with the advancement in hydration, the respiratory activity becomes limited by decarboxylation and phosphorylative capacity (Weitbrecht *et al.*, 2011).

The intense respiratory activity in the first hours of imbibition is necessary for the generation of the redox state (Rosental *et al.*, 2014) and the energetic requirement is directed to the production of systems of removers of reactive forms of oxygen in seeds of lower physiological potential submitted to supra-optimal temperatures (Dantas *et al.*, 2015).

According to Figure 1, the reduction in the concentration of CO₂ released from wheat seeds is noticeable as a function of the reduction in temperature, and after 24 h of incubation at 15 °C, the reduction in the CO₂ release is three times lower than that observed at 40 °C. Modulation in the enzymatic kinetics is directly proportional to the temperature at which seeds are found (Bandeira *et al.*, 2013), besides reflecting the thermal

Table 1: Percentage of normal seedlings in germination test (NS), first germination count (FC), dry mass of seedlings (PDM), potassium leaching (KL), electrical conductivity (EC) and field emergence (FE) of six lots of *Triticum aestivum* CD 123 seeds

Lot	NS	FC	PDM	KL	EC	FE
	%	%	mg ⁻¹	μmol g ⁻¹	— μS cm g ⁻¹	%
1	94.0b	91.0b	21.55c	188.50a	42.22a	91.0c
2	97.0a	93.5b	22.54b	182.96a	39.90a	93.0b
3	98.0a	92.5b	22.52b	185.66a	38.04b	94.5b
4	97.0a	94.5a	23.64a	171.07b	38.87b	93.5b
5	97.0a	96.0a	23.67a	156.64b	36.63b	98.5a
6	99.0a	95.0a	23.45a	73.82c	23.85c	97.5a
CV (%)	1.8	1.9	1.9	7.2	5.1	1.8

Means followed by the same lowercase letter in the column do not differ statistically from each other at the level of 5% of error probability by the Scott-Knott test.

requirements necessary for the occurrence of root production (Patanè & Avola, 2013).

Therefore, the objective of temperature manipulation in the evaluation of respiratory activity is to expose the seeds to adverse conditions from that considered optimum, since the ability of seeds to germinate under a broad thermal limit is a manifestation of their vigor. Therefore, seeds that tolerate a greater thermal variation are the most vigorous and susceptible to segregation by respiratory activity.

It was possible to separate lots of wheat seeds at three vigor levels after 24 h of incubation at 15 °C. The composition of groups occurred similarly to that observed after 12 and 24 h of incubation at 25 °C (Table 2). The incubation at 40 °C allowed the separation of the lot with lower physiological potential, therefore, when the objective is seed vigor evaluation, the use of this temperature is not recommended since it was not possible to separate lots of higher physiological potentials from the intermediary vigor.

It is also stressed that the composition of the groups arranged after 24 h at 15 °C and after 12 h of incubation at 25 °C, the similarity of the observed one to field emergence (Table 1), whose lots with the highest emergency rate released a CO₂ 14% lower than the lot with reduced physiological potential, as well as the average values obtained after 24 h of incubation at 15 °C were similar to those obtained with a 12 h incubation at 25 °C, reducing by half the period of time necessary to separate the lots at different levels of vigor.

The simple correlation analysis between the values obtained from the CO₂ concentration and the percentage of normal seedlings emerged in the field revealed the

existence of a significant negative correlation ($p < 0.05$) from 9 h of incubation, when incubated at 15 °C (Table 2). For samples incubated at 25 °C, correlation coefficients lower than -0.85 were obtained after three hours of incubation. These results indicate that field emergence was inversely proportional to the concentration of the released CO₂. Therefore, seed lots that release less CO₂ per unit of mass are the ones with the best performance in the field. For samples incubated at 40 °C, no significant correlation was found.

The increase in CO₂ content released as a function of loss of viability is linked to the structural changes in the biological membranes caused by lipid peroxidation, which influence the viscosity, permeability and cellular functions of the mitochondria (Shaban, 2013). Therefore, the passage from the dry state to the fluid of the cell membranes promotes the rapid diffusion and capillarity of the water in aged seeds, and the advance in the faster absorption of water causes the mitochondria present in dry seeds to resume its metabolic activity more quickly, resulting in a higher CO₂ release in wheat seeds, as it can be illustrated by the correlation coefficient of 0.83 ($p = 0.0321$) between the values obtained by the electrical conductivity test with the CO₂ concentration released after 12 h of incubation at 25 °C.

Attention should be given to the fact that the germination speed and the seedling growth rate were inversely proportional to the content of CO₂ released, considering that the simple correlation coefficients were -0.92 and -0.88 ($p < 0.05$), respectively, which corroborates with those reported by Rosental *et al.* (2014), whose respiratory activity is indicative of vigor and its reduction directly affects the speed and growth rate of the seedlings.

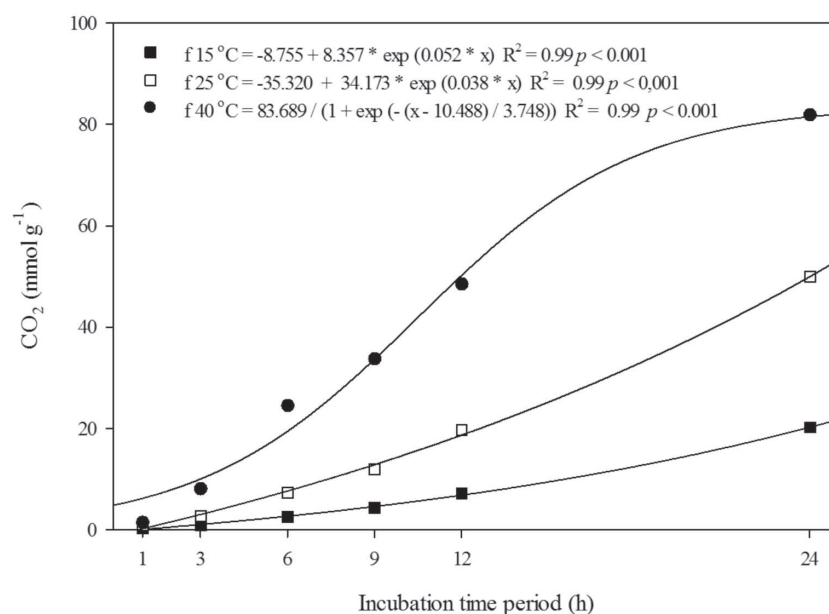


Figure 1: Concentration of CO₂ released from *Triticum aestivum* CD 123 seeds as a function of time and incubation temperature.

The high coefficients of correlation between the concentration of CO₂ obtained with the percentage of emerged seedlings suggest that the methodology was efficient in the prediction of the establishment of seedlings in the field, however, temperatures lower than or equal to 25 °C allow the greater separation of the lots, resulting in greater sensitivity in detecting differences in physiological potential in *Triticum aestivum* seeds not detected by viability tests, therefore, with the potential to classify lots according to their performance potential.

Validation of the analytical method

The test of Grubbs applied in twenty replicates for the fifteen lots did not detect the presence of extreme values (Figure 2a). This test verifies the existence of an atypical value at each end of the set (Chui *et al.*, 2009). Extreme values can be considered as a natural variation in a set of data or even by errors of measurement, execution, and operation.

Evaluation of accuracy by comparing the critical limit of repeatability (CLR) indicated an acceptable repeatability for the 15 tested lots since results of individual total amplitude remained below the calculated critical limit at a significance level of 1% (Figure 2b). These results express the degree of agreement between the results of successive measurements of the same measurand (represented by seed lots) carried out under the same predetermined measurement conditions.

According to the analysis of variance, there was no evidence of significant differences in the obtention of the concentration of CO₂ in different working days (Table 3), which indicates precision in the achievement of the results for the 15 lots of wheat seeds, since even with the changing in the shifts, the method allowed to obtain statistically identical results.

The results for the accuracy analysis showed agreement between the quantification by the infrared gas analyzer and that obtained by gas chromatography

Table 2: Concentration of CO₂ released from *Triticum aestivum* CD 123 seeds as a function of the lot and incubation time and its correlation (r) with the percentage of the emergence of seedlings in the field

Temperature (°C)	Lot	Incubation time period (h)					
		1	3	6	9	12	24
		mmol g ⁻¹					
15	1	0.30a	0.90a	2.62a	4.74a	7.33a	21.73a
	2	0.31a	0.94a	2.46a	4.45a	7.43a	21.00b
	3	0.34a	0.86a	2.51a	4.19a	7.18a	20.67b
	4	0.29a	0.88a	2.77a	4.22a	7.23a	20.21b
	5	0.28a	0.94a	2.85a	4.10a	6.79b	18.95c
	6	0.20a	0.53a	2.19a	4.03a	6.73b	18.55c
CV (%)		9.17					
r		-0.54	-0.40	-0.03	-0.89**	-0.92**	-0.95**
25	1	0.83a	2.95a	7.88a	12.46a	21.54a	52.31a
	2	0.93a	2.76a	7.62a	12.10a	20.17b	50.66b
	3	0.79a	2.59a	7.39a	11.93a	20.15b	49.28b
	4	0.76a	2.69a	7.06a	11.86a	19.98b	50.43b
	5	0.88a	2.55a	7.03a	11.77a	18.54c	48.56c
	6	0.48a	2.42a	6.95a	11.48b	18.62c	47.77c
CV (%)		6.49					
r		-0.37	-0.91**	-0.85**	-0.87**	-0.95**	-0.94**
40	1	1.34a	7.51a	20.29a	35.18a	53.54a	91.25a
	2	1.43a	6.85a	20.16a	34.93a	50.04a	87.58b
	3	1.44a	7.55a	19.93a	35.72a	52.58a	86.21b
	4	1.45a	6.94a	19.37a	33.98a	49.99a	84.88b
	5	1.32a	6.87a	19.34a	35.12a	49.72a	85.76b
	6	1.12a	7.17a	20.56a	34.41a	48.60a	83.20b
CV (%)		26.10					
r		-0.49	0.18	-0.53	-0.34	-0.13	0.10

Means followed by the same lowercase letter in the column do not differ statistically from each other at the level of 5% of error probability by the Scott-Knott test. ** Significant at 5% of error probability by the test t.

(reference method), considering that no significant differences ($p > 0.05$) were detected between the methods by t test (Table 4). Also, the correlation analysis showed a simple correlation coefficient of 0.97 ($p < 0.0001$) suggesting a linear and directly proportional relationship to that obtained by gas chromatography.

The results obtained for normal seedlings indicated that among the fifteen seed lots, it was possible to form two feasible groups, with germination percentage of 98 and 95%, respectively. The group that presented a higher germination rate resulted in the formation of four groups

for the concentration of CO_2 released, with averages ranging from 22.39 to 18.46 $\text{mmol g}^{-1} \text{CO}_2$, outlining the sensitivity of the method to detect differences in the physiological potential in seeds with similar germination. It is also observed the tendency of reduction in the release of CO_2 as the germinative rate increased, considering that the inverse relation between the tests resulted in a correlation coefficient of -0.93 ($p < 0.0001$).

Additionally, the methodology proposed in the present study can be widely used in the quality control of seed production, processing, and storage in wheat seed

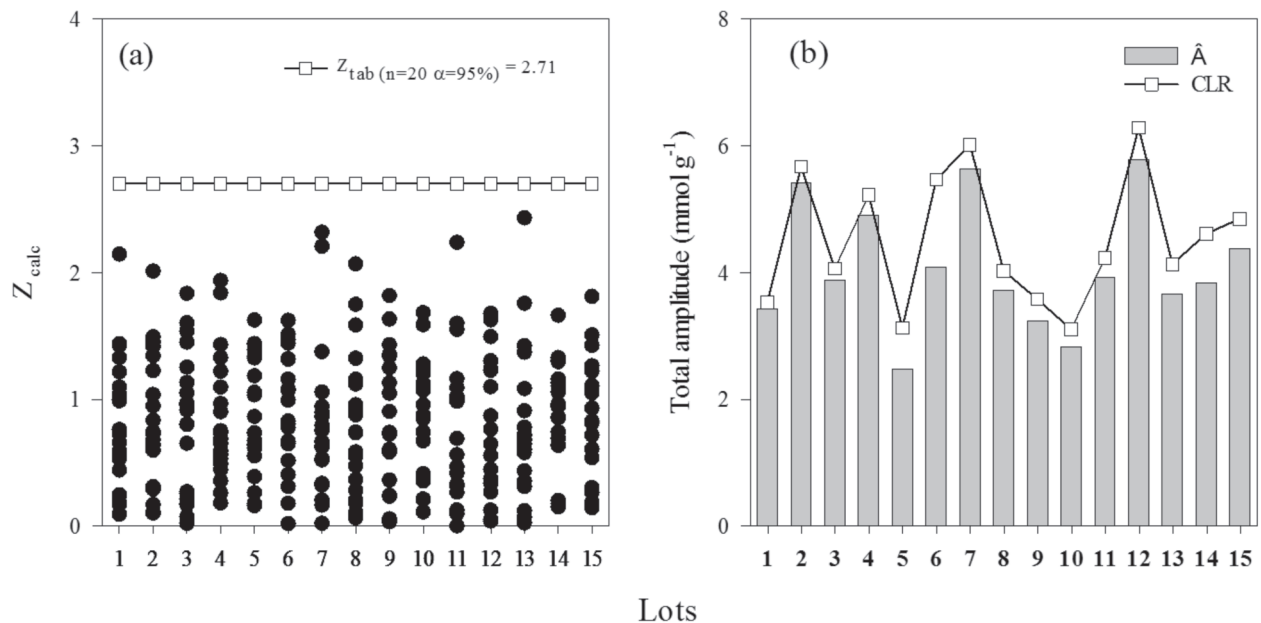


Figure 2: (a) Extreme value test (Grubbs' test) applied to the results of the CO_2 concentration in fifteen seed lots of *Triticum aestevium* CD 123. (b) Precision analysis by comparing critical limit of repeatability (CLR) with total amplitude (\hat{A}).

Table 3: Repeatability evaluation by comparing days of analysis per lots of *Triticum aestevium* CD 123 seeds.

Lot	First day (n=10)	Second day (n=10)	ANOVA		
	mmol g^{-1}	Q_{Mdia}	F_{calc}		P
1	24.95 ± 1.22	24.74 ± 0.72	0.229	0.227	0.639
2	23.71 ± 1.64	25.10 ± 1.23	9.638	3.113	0.065
3	23.71 ± 1.09	23.99 ± 1.21	0.406	0.306	0.587
4	23.75 ± 1.57	24.00 ± 1.40	0.306	0.138	0.714
5	23.43 ± 0.74	23.91 ± 0.96	1.142	1.549	0.229
6	23.37 ± 1.22	23.87 ± 1.61	1.218	0.600	0.449
7	22.31 ± 1.50	22.82 ± 1.88	1.287	0.445	0.513
8	22.59 ± 1.09	22.53 ± 1.20	0.021	0.016	0.902
9	21.98 ± 0.94	21.64 ± 1.07	0.584	0.577	0.457
10	21.04 ± 0.93	21.21 ± 0.84	0.149	0.191	0.668
11	20.86 ± 1.21	21.32 ± 1.16	1.043	0.741	0.401
12	20.29 ± 1.91	21.29 ± 1.50	4.980	1.686	0.211
13	20.19 ± 1.24	20.25 ± 1.12	0.019	0.014	0.908
14	19.42 ± 1.15	20.13 ± 1.37	2.557	1.600	0.222
15	17.97 ± 1.41	18.94 ± 1.16	4.691	2.821	0.110

Table 4: Accuracy analysis by comparison of the CO₂ concentration by the reference method with the proposed method, by simple correlation (R) and comparison of means of the proposed method and percentage of normal seedlings (PN) of 15 lots of *Triticum aestivum* CD 123.

Lot	Reference method	Proposed method	PN	$\rho_{\text{test t}}$
	(n = 4)	(n = 20)		
	mmol g ⁻¹		%	
1	25.69 ± 1.73	24.84 ± 0.98 a	94 b	0.18
2	24.77 ± 2.70	24.40 ± 1.58 a	95 b	0.71
3	23.32 ± 1.48	23.85 ± 1.13 b	96 b	0.46
4	22.96 ± 1.71	23.78 ± 1.45 b	95 b	0.45
5	23.72 ± 0.49	23.63 ± 0.81 b	96 b	0.83
6	23.43 ± 2.39	23.62 ± 1.41 b	96 b	0.36
7	21.94 ± 2.51	22.39 ± 1.49 c	97 a	0.63
8	22.55 ± 2.26	22.35 ± 1.21 c	98 a	0.80
9	21.31 ± 0.70	21.81 ± 1.00 d	98 a	0.36
10	20.79 ± 1.26	21.13 ± 0.86 d	98 a	0.51
11	20.55 ± 0.97	20.94 ± 1.18 d	98 a	0.40
12	21.45 ± 0.24	20.68 ± 1.75 d	99 a	0.46
13	19.87 ± 1.71	20.12 ± 1.15 e	99 a	0.60
14	18.42 ± 0.70	19.59 ± 1.29 e	99 a	0.59
15	19.13 ± 0.45	18.46 ± 1.35 f	99 a	0.34
R	0.97 $p < 0.0001$			

Where: $\rho_{\text{test t}}$ and p = probability of significance by the two-tailed t test. Means followed by the same lowercase letter in the column do not differ statistically from each other at the level of 5% of error probability by the Scott-Knott test.

industries, as well as in quantifying the respiratory activity of biological organisms that result in the release of CO₂ by oxidative and fermentative processes.

CONCLUSIONS

For the evaluation of the respiratory activity in *Triticum aestivum* seeds using CO₂ concentration, it is recommended to incubate the samples at 25 °C for a minimum period of 12 hours, which allows to classify lots with different levels of vigor and to predict the establishment of seedlings in the field. Based on the intra-laboratory validation, this methodology is adequate for the measurement of CO₂ in seeds, since externalize precision between successive measurements and agreement with the reference method.

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