



Research article

Prediction of groundnut (*Arachis hypogaea* L.) seed quality after ethanol vapor ageing based on seed respiration rate using non-dispersive infrared CO₂ sensor

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Abstract

Importance of the work: Conventional methods for evaluating groundnut seed quality are time-consuming and often destructive. Real-time respiration measurement could provide a rapid and non-destructive method for assessing seed viability and vigor.

Objectives: To generate groundnut seed lots with varying physiological quality through ethanol vapor ageing, determine the relationship between seed quality and respiration rate, and develop a real-time respiration rate measurement method for indicating seed viability and vigor.

Materials and Methods: Groundnut seed lots with varying physiological quality were generated by ethanol vapor ageing for 0 min, 10 min, 20 min, 30 min or 40 min. Seed viability and vigor were assessed, and real-time respiration rates were measured for 24 hr using a non-dispersive infrared CO₂ sensor to determine their relationship with seed physiological quality.

Results: Ethanol vapor ageing progressively reduced seed quality, with germination decreasing from 88.5% to 6.0%, tetrazolium viability from 84.0% to 26.0%, and electrical conductivity increasing from 4.69 to 51.02 $\mu\text{S}/\text{cm}/\text{g}$ as ageing duration increased from 0 min to 40 min. Seed respiration rate was significantly and negatively correlated with physiological quality over 24 hr, and the 3 hr measurement produced strong predictive relationships with all quality parameters ($R^2 > 0.80$), including germination ($R^2 = 0.88$) and tetrazolium viability ($R^2 = 0.97$), indicating its potential as a rapid, non-destructive test.

Main finding: Ethanol vapor ageing in a vacuum container effectively generated groundnut seed lots with varying levels of physiological quality. Real-time respiration rate, particularly after 3 hr, showed potential as a rapid and non-destructive indicator of seed viability and vigor.

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Introduction

Groundnut (*Arachis hypogaea* L.) seeds have a high nutritional content, specifically of fat (49%) and protein (27%) (Bonku and Yu, 2019). However, the seeds cannot be stored for long periods because of these high contents, since inappropriate storage conditions accelerate the decline in seed quality such as storing seed at high temperature, high relative humidity and high seed moisture content (Gayathri et al., 2024; Raja, 2025). During seed storage, particularly under non-ideal conditions, seeds undergo oxidative stress due to accelerated metabolic processes, characterized by the increased accumulation of reactive oxygen species (ROS). This elevation in ROS oxidizes lipids and amino acids, leading to damage in seed cells and seed metabolism (Ramtekey et al., 2022). ROS induce lipid peroxidation, which attacks unsaturated fatty acids and affects membrane permeability, damaging various organelles, notably the seed mitochondria (Ebene et al., 2019; Li et al., 2022). Furthermore, the increase in ROS during storage modifies proteins, resulting in damage and degradation known as protein carbonylation. Structural changes in proteins can occur within various cells, specifically targeting the mitochondria (Zhang et al., 2021). The use of low-quality seeds can result in poor and slow seedling emergence in the field, making the plants susceptible to disease (Jasim and Esho, 2022; Reed et al., 2022).

Frequently, respiration activity is adopted as a rapid test due to its established correlation with a seed's germination potential. Other studies have utilized various metabolic indicators related to respiration, including dehydrogenase enzyme activity (Costa et al., 2018; International Seed Testing Association, 2018; Sales et al., 2022), respiration rate quantified based on oxygen consumption (Bello and Bradford, 2016) and on carbon dioxide release (Widajati et al., 2023) and adenosine triphosphate (ATP) concentration (Spoelstra et al., 2002). Based on CO₂ release, the respiration rate has strong potential as a rapid, non-destructive and simple method. The CO₂ respiration rate can be detected using either titration techniques or CO₂ detection sensors. The titration method is conducted by incubating seeds within a sealed container for several days. Seed are placed on moist paper, while the container holds a beaker of solution capable of binding with CO₂ such as KOH and Ba(OH)₂ (Dode et al., 2016; Jawak et al., 2022). Subsequently, this solution is titrated to calculate the quantity of CO₂ produced by the seed respiration rate. However, this method relies on the analytical precision to obtain accurate and precise results

during the titration process and is time consuming. The use of a sensor provides more precise, accurate and objective results compared to titration. Furthermore, sensors allow for real-time data recording, enabling the continuous measurement of respiration activity (Dalziel and Tomlinson, 2017; Jiang et al., 2018; Ruiz-Nieves et al., 2021). Sensors can detect CO₂ by measuring the amount of infrared (IR) radiation absorbed at specific wavelengths, notably 2.0 µm, 2.7 µm, 4.3 µm and 15.0 µm (Xu et al., 2024; Ng et al., 2025). However, the correlation between seed quality and CO₂ respiration rate is highly diverse across different commodities. Some studies have reported a positive correlation between seed quality and respiration rate (Dalziel and Tomlinson, 2017; Ruiz-Nieves et al., 2021), while others identified a negative correlation (Dode et al., 2016; Horbach et al., 2017). Several studies have detected seed quality through a single-point respiration rate measurements conducted at a certain time (Jawak et al., 2022; Rodrigues et al., 2022). However, continuous real-time monitoring is required to identify a stable metabolic plateau, which provides more precise information regarding respiratory activity.

In developing a reliable method for indicating seed quality, it is essential to have a diverse set of seed groups (lots) exhibiting a variation in physiological quality. Achieving this requires variation in physiological quality that can be achieved through natural long-term storage (Li et al., 2025). However, the natural deterioration process is time consuming. Consequently, the creation of seed lots with varied physiological quality can be accomplished more efficiently using an accelerated ageing method based on seed longevity vigor principles (Ebene et al., 2019; Wang et al., 2023). Seed longevity vigor refers to the seed's capacity to survive under long-term or suboptimal storage conditions. Seeds with high longevity vigor have the ability to maintain optimal germination following a period of adverse storage. Longevity vigor can be evaluated through simulated storage tests (accelerated ageing), involving environmental conditions such as high temperature and high humidity (Fenollosa et al., 2020; Srii and Nagarajappa, 2024). Furthermore, a high moisture content can be used to manipulate seed condition (International Seed Testing Association, 2018). Additionally, chemical agents (specifically ethanol), can be utilized effectively in evaluating seed storage vigor.

Mitochondrial damage during storage causes seeds to shift their respiratory activity from aerobic to anaerobic pathways; a transition marked by the increased accumulation of endogenous ethanol (Kucukhuseyin et al., 2021). This build-up of high ethanol levels can damage seed cells and metabolic activities. For example, several studies have utilized ethanol

to accelerate the deterioration process, with the seeds being exposed to ethanol through either vapor or soaking (Taini et al., 2019; Amanah et al., 2016), resulting in a rapid increase in endogenous ethanol levels. Ethanol induces structural changes in cell membranes and oxidative stress due to elevated levels of ROS. These processes disrupt mitochondrial metabolism, ultimately leading to a decline in seed quality (Zhang et al., 1994; Toth et al., 2014; Butnor et al., 2018; Chen et al., 2023).

The current study was conducted with three primary aims: 1) to obtain a series of diverse seed lots with variation in their physiological quality through ethanol vapor ageing in a vacuum container; 2) to establish the relationship between seed physiological quality and its respiration rate; and 3) to develop a real-time respiration rate measurement method capable of indicating seed physiological quality (viability and vigor).

Materials and Methods

The experiment was conducted at the Seed Science and Technology Laboratory, Department of Agronomy and Horticulture, IPB University, Bogor, Indonesia during June–September 2025. The study utilized the Kelinci variety of groundnut seed, sourced from Badan Peraktikan dan Modernisasi Pertanian Aneka Kacang, Malang, Indonesia.

Experiment 1: Generation of seed lots based on ethanol vapor ageing in vacuum container

This study was designed as a single factor experiment using a randomized block design (RBD). The initial seed lot was divided equally into five sub-lots, each of which was subjected to a different ethanol vapor ageing treatment (0 min, 10 min, 20 min, 30 min, 40 min). Due to instrumental limitations, each replicate was conducted on a different day. Therefore, the different working days were treated as a blocking factor.

Initially groundnut seeds (moisture content (MC) 5.7%) were moistened by placing them between layers of damp paper for 12 hr at $25 \pm 2^\circ\text{C}$ (MC 18.0%). Then, the seeds were placed on a wire mesh suspended above a jar holding 100 mL of 96% ethanol (Fig. 1). Subsequently, the seeds were placed inside a sealed container (1,300 mL Bosman acrylic vacuum chamber) and subjected to a vacuum (Bosman vacuum pump; Bosman; Indonesia) for 1 min. This step was performed to facilitate the rapid displacement of interstitial air to ensure uniform contact between the ethanol vapor and the seeds. The seeds were incubated in the saturated ethanol atmosphere for 0 min, 10

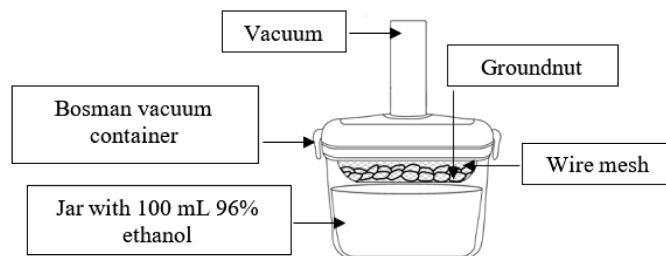


Fig. 1 Schematic illustration of the ethanol vapor ageing system using a vacuum container.

min, 20 min, 30 min, or 40 min. Following the ageing process, the treated seeds were air dried at room temperature ($29 \pm 2^\circ\text{C}$) for 1 d until the seed moisture content reached approximately 12%.

The initial seed MC was determined using the air oven method at $103 \pm 2^\circ\text{C}$ for 17 hr (International Seed Testing Association, 2018). Subsequent changes in the MC during the moistening and air-drying processes were monitored non-destructively using the constant-weight method (International Seed Testing Association, 2018), according to Equation 1:

$$W_f = W_i (100 - MC_i) / (100 - MC_f) \quad (1)$$

where W_f is the targeted seed weight, W_i is the initial seed weight, MC_i is the initial moisture content and MC_f is the targeted moisture content (International Seed Testing Association, 2018).

Physiological quality analysis

Germination was evaluated using the between paper method for a period of 10 d at a constant temperature of $25 \pm 2^\circ\text{C}$ (International Seed Testing Association, 2018), based on a sample size of 50 seeds for each seed lot.

The germination (G) percentage was observed on day 5 and day 10 after sowing, based on the number of normal seedlings produced, according to Equation 2:

$$G = (\sum \text{Normal seedlings}) / (\sum \text{Seeds}) \times 100 \quad (2)$$

First count germination (FC) was observed on day 5 based on the number of normal seedlings produced, according to Equation 3:

$$FC = (\sum \text{Normal seedlings}) / (\sum \text{Seeds}) \times 100 \quad (3)$$

The maximum germination potential (MGP) was observed on day 10, accounting for all seeds that germinated into both normal and abnormal seedlings, according to Equation 4 (Gundala et al., 2023):

$$\text{MGP} = (\sum \text{Germinated seeds}) / (\sum \text{Seeds}) \times 100 \quad (4)$$

The germination rate index (GRI, measured as a percentage) per day, was observed every day until day 10 after sowing, according to Equation 5 (Kader, 2005):

$$\text{GRI} = \sum ((\text{GP}_1/1) + (\text{GP}_2/2) + \dots + (\text{GP}/10)) \quad (4)$$

where GP_1 is the germination percentage on the first day after sowing, GP_2 is the germination percentage on the second day after sowing through to GP_{10} being the germination percentage on the tenth day after sowing. The daily germination percentage results were accumulated until day 10.

Electrical conductivity (EC, measured in micro siemens per centimeter per gram) was determined using a sample of 10 g of groundnut seed with 12% moisture content that had been soaked in 40 mL of deionized water for 12 hr at $25 \pm 2^\circ\text{C}$ (Suganthi and Selvaraju, 2017). The seeds were separated from the soaking water, which was then measured using an electrical conductivity meter. The conductivity of the seed-soaking water was compared with that of the pure deionized water, according to Equation 6:

$$\text{EC} = (\text{EC}_{\text{sample}} - \text{EC}_{\text{deionized water}}) / \text{Seed weight} \quad (6)$$

where $\text{EC}_{\text{sample}}$ is the EC of the sample soaking water and $\text{EC}_{\text{deionized water}}$ is the EC of the deionized water and the seed weight is measured in grams.

The percentage viability test using tetrazolium (TZ) used a modified version of the procedure described by Santos et al. (2012). A sample of 100 groundnut seeds was soaked in distilled water for 18 hr. After the seed coats had been removed, the cotyledons were opened without being separated. Then, the seeds were then soaked in 0.2% tetrazolium solution for 4 hr at 40°C . Subsequently, the seeds were evaluated based on their staining pattern and categorized as viable or non-viable seeds. Tz was determined according to Equation 7:

$$\text{TZ} = (\sum \text{Viable seeds} / \sum \text{Seeds}) \times 100 \quad (7)$$

Experiment 2: Seed physiological quality indication based on respiration rate

This study was designed as a single factor experiment using a RBD with four replicates. The factor investigated was the various seed lots, representing a range of physiological quality levels, as obtained in Experiment 1. Due to instrumental limitations, each replicate was conducted on a different day. Therefore, the different working days were treated as a blocking factor.

Respiration rate

The CO_2 respiration rate was measured using a non-dispersive infrared (NDIR) CO_2 sensor (Lutron CO_2 -9904 SD; Lutron Electronic Enterprise Co. Ltd. ; Taiwan). A sample of 75 g of groundnut seed, maintained at a seed moisture content of 12%, was placed inside a customized acrylic container (7 cm $\times$ 7 cm $\times$ 30 cm) equipped with the NDIR CO_2 sensor (Fig. 2). The container was hermitically sealed using petroleum jelly to ensure an airtight environment.

The CO_2 concentration was recorded every 15 min during 24 hr. The resulting data were processed and plotted as a graph, where the x-axis represented time (measured in hours) and the y-axis represented the change in CO_2 concentration (measured on a percent volume per volume basis), which was used to derive a regression equation (Equation 8):

$$y_{\text{CO}_2} = ax + b \quad (8)$$

where a is the slope of the graph and b is the y intercept.

The CO_2 respiration rate (Rr , measured in milligrams of CO_2 per kilogram of dry mass per hour) was calculated for every 3 hr interval using Equation 9 (Ochandio et al., 2017):

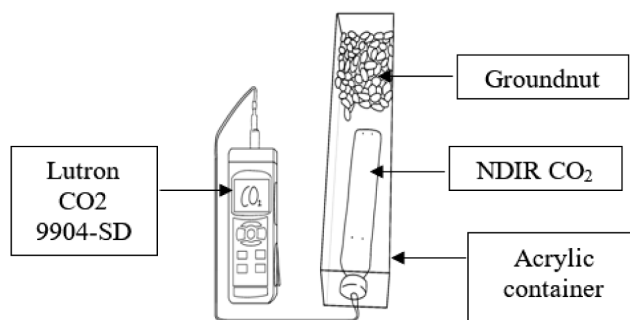


Fig. 2 Schematic illustration of groundnut seed respiration rate measurement using a non-dispersive infrared CO_2 sensor (NDIR CO_2).

$$Rr = (1/100) \times P \times V \times (M_{CO_2}/(R \times T) \times (1/DM) \times (d[CO_2]/dt) \quad (9)$$

where P is the atmospheric pressure (101,325 Pa), V is the air free volume in 1 m³ (0.0011 m³), M_{CO₂} is the molar mass of the CO₂ (44.010 mg/mol), R is the ideal gas constant (8.314 J/K/mol), T is the temperature (298.15K), DM is the seed dry mass in kilograms and (d[CO₂]/dt) is the first derivative of the linear regression (Equation 9).

Decline in respiration rate

The seed respiration rate against the measurement duration was initially assumed to follow an exponential growth curve. To simplify the analysis of the differing rates of decline in respiration (DR_r), the resulting curve was transformed into a linear graph using the natural logarithmic (ln) method, according to Equation 10:

$$\ln(Rr(t)/Rr(0)) = \ln(e^{-kt}) \quad (10)$$

where Rr(t) is the respiration rate (Rr) at the measurement duration t (measured during 3–24 hr), Rr(0) is the initial Rr at 3 hr, t is the measurement duration in hours and k is the constant rate of decline. The subsequent equation was simplified further into the Equation 11:

$$y = -kt \quad (11)$$

y is the natural logarithm of the respiration rate $\ln(Rr(t)/Rr(0))$, k is the gradient (slope) of the line and t is the duration, measured in hours.

Data analysis

Analysis of variance was conducted on the ethanol ageing

duration treatments. Subsequently, the honestly significant difference test was applied at a significance level of $\alpha = 5\%$ to compare treatment means. Correlation and regression analysis between the respiration rate and seed quality were conducted using the data obtained from the optimal respiration rate measurement duration. All statistical analysis were performed using R, a language and environment for statistical computing and graphics (version 4.1.1; R Core Team, 2021).

Results and Discussion

Experiment 1: Generation of seed lots based on ethanol vapor ageing in vacuum containers

Experiment 1 was designed to generate diverse seed lots having varying levels of physiological quality (seed viability and vigor) based on ethanol vapor ageing. Based on the results, the ethanol vapor ageing method in a vacuum container was effective in reducing seed quality (Table 1). There was a gradual decline in seed germination performance (based on GP, FC and MGP), corresponding to an increased duration of ethanol vapor ageing. Furthermore, cellular damage within the seeds was suggested by the elevated EC values that indicated increased seed membrane leakage that was hypothesized to stem from membrane deterioration potentially induced by free radical activity. Mandizvo and Odindo (2019) reported that the process of physical accelerated ageing could cause direct damage to the cell membrane structure due to the enhanced production of free radicals. Correspondingly, Mubarak et al., (2025) stated that groundnut seeds with low viability had high protein leakage, a phenomenon negatively correlated with overall seed viability.

Table 1 Physiological quality of groundnut seeds after ethanol vapor ageing.

AD (min)	G (%)	MGP (%)	FC (%)	GRI (%/d)	EC (μS/cm/g)	TZ (%)
0	88.5±5.0 ^a	98.5±1.0 ^a	18.5±3.0 ^a	15.2±0.4 ^a	4.69±0.4 ^c	84.0±1.8 ^a
10	90.0±4.9 ^a	98.0±1.6 ^a	16.0±8.3 ^a	14.9±0.7 ^a	5.49±0.6 ^c	78.5±4.4 ^a
20	67.5±15.7 ^a	92.5±6.2 ^a	11.0±4.8 ^{ab}	11.3±1.8 ^b	15.05±0.6 ^c	68.8±4.6 ^b
30	27.5±12.3 ^b	67.0±17.4 ^{ab}	2.5±1.9 ^{bc}	3.3±2.0 ^c	33.61±7.7 ^b	55.0±5.1 ^c
40	6.0±6.7 ^b	39.0±23.6 ^b	0.5±1.0 ^c	1.4±1.3 ^c	51.02±10.2 ^a	26.0±2.9 ^d
p value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
CV (%)	18.4	18.4	26.4	15.8	8.8	4.9

AD = ethanol vapor ageing duration; CV = coefficient of variation; EC = electrical conductivity; FC = first count germination; G = germination; GRI = germination rate index; MGP = maximum germination potential; TZ = tetrazolium viability test.

Mean ± SD within the same column followed by different lowercase superscript letters are significantly ($p < 0.05$) different, based on honestly significant difference analysis.

The ethanol vapor ageing served as an accelerated proxy for the natural seed storage process. While ethanol represented a direct chemical stress, it induced physiological disruptions (specifically a decline in both seed viability and vigor) that paralleled the metabolic shifts observed during extended storage periods (Chaengsakul et al., 2019; Barbosa et al., 2021). This treatment provided rapid simulation of the endogenous ethanol accumulation typically associated with mitochondrial decay in naturally aged seeds. The exposure to exogenous ethanol enhanced the ethanol concentration within the seeds. Consequently, germination capacity of the seeds was diminished. Ethanol exposure inhibits seed germination by suppressing the expression of ethylene receptor genes (Chen et al., 2020) and SOD enzymes, while simultaneously increasing ABA and decreasing GA levels (Chen et al., 2023). Ethanol causes cellular damage by denaturing proteins and binding to membrane lipid components, thereby increasing fluidity and inducing electrolyte leakage, which reduces seed viability (Toth et al., 2014; Amanah et al., 2016).

In general, seed viability (TZ) and vigor (FC, GRI, EC) began to decline following 20 min of ethanol vapor ageing. Based on this ageing method, the various seed lots represented a range of physiological quality levels obtained in Experiment 1. These lots had a spectrum of physiological quality, categorized as high vigor lots (0 min and 10 min ageing), a medium vigor lot (20 min ageing), a low vigor lot (30 min ageing) and a very low vigor lot (40 min ageing).

Experiment 2: Seed physiological quality indication based on respiration rate

Experiment 2 was designed to investigate the correlation between the seed respiration rate and the level of seed physiological quality. The samples used in this experiment were the four seed lots with varying vigor levels successfully generated in experiment 1, namely seed lots with high vigor (0 min ageing), medium vigor (20 min ageing), low vigor (30 min ageing) and very low vigor (40 min ageing). Based on the results from the 24 hr respiration rate measurements, there were significant differences among the seed lots. Across the entire measurement duration, a consistent pattern emerged, with the lower seed vigor having a higher respiration rate (Fig. 3).

All seed lots had a decline in the respiration rate as the measurement duration increased, following an exponential curve. Subsequently, the rate of decrease in seed respiration was determined by transforming the exponential graph into a linear plot using the natural logarithmic method. The seed lots displayed distinct patterns of respiratory behavior, particularly comparing the high vigor lot with the medium-to-very-low vigor lots. Uniquely, the high vigor lot showed an increase rather than a decline in respiration rate during the initial 6 hr, with the decline in the respiration rate for this lot only becoming evident during 6–24 hr. Conversely, the medium-to-very-low vigor lots showed a consistent decrease in the respiration rate throughout the 3–24 hr period. Crucially, the high vigor lot

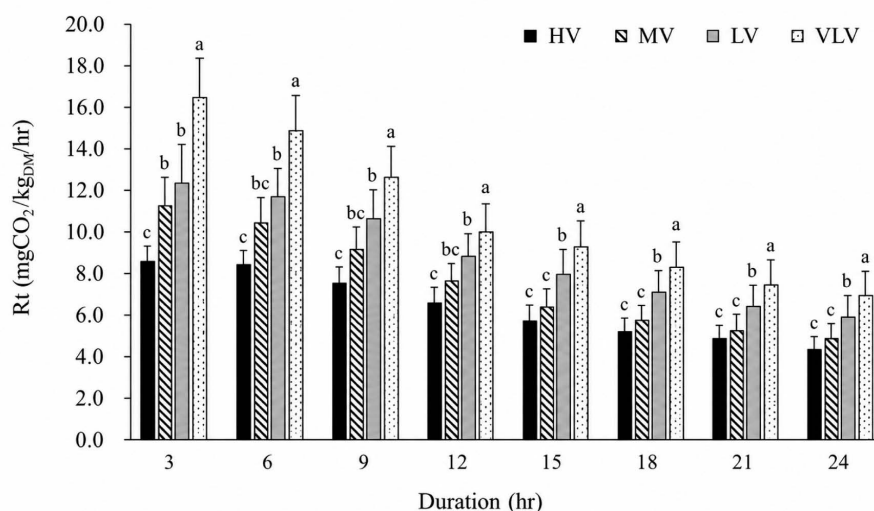


Fig. 3 Respiration rate (Rr) of groundnut seed lots during 24 hr of HV (high vigor lot), MV (medium vigor lot), LV (low vigor lot) and VLV (very low vigor lot). Different lowercase letters above columns, at the same duration point, indicate significant difference ($p < 0.05$) among seed lots based on honestly significant difference analysis. DM = dry mass. Error bars indicate $\pm$ SD.

had a significantly slower rate of decline in respiration than the medium-to-very-low vigor lots, while the medium vigor seed lot had a respiration rate that was not significantly different from both the high vigor and low vigor lots for several specific measurement durations (Fig. 3), although it had the sharpest overall decline in respiration rate (Fig. 4). This volatility strongly suggested that the metabolism within the medium vigor lot was highly unstable.

The high respiration rate in the lower-quality seed lots, as well as the decline in the respiration over time, may have been due to ethanol-induced accelerated ageing. This process acts as a chemical stressor that hastens seed deterioration through alteration in cellular structure, metabolic inhibition and the induction of oxidative stress. Woodstock and Taylorson (1981) reported an imbalance between glycolysis and the tricarboxylic acid (TCA) cycle in seeds subjected to oxidative stress. In such cases, glycolysis proceeds rapidly while the TCA cycle operates more slowly, leading to the accumulation of pyruvic acid. This excess pyruvate is diverted towards anaerobic respiration, which releases considerable amounts of CO₂. Furthermore, Tiwari et al. (2002) stated that oxidative stress caused mitochondrial damage. Seed experiencing oxidative stress exhibit symptoms of uncoupling respiration, characterized by increased respiratory activity that is not accompanied by ATP synthesis, but rather by the formation of ROS. Consequently, seed lots exposed to ethanol show high respiration only during the initial measurements, with a subsequent decline due to the progressive damage caused by ROS accumulation.

The correlation analysis result confirmed that the seed respiration rate, measured in real time over during 3–24 hr,

had a significant, negative correlation with all the established measures of physiological quality (Fig. 5). Based on the strength of this correlation analysis and the observed respiration rate data (Fig. 3), the 3 hr respiration rate measurement was selected for further regression analysis. This duration was chosen because it had a significant difference in respiration rate among the seed lots, maintained a significant correlation with the quality metrics and represented the shortest practical measurement duration for a rapid test. Based on the subsequent results, the real-time 3 hr respiration rate measurement could serve effectively as an indicator for both seed viability and vigor. This indicative power was supported by the R² values (> 0.80) for all the physiological quality metrics (Fig. 6). While this finding differed from several published reports, comparable studies do exist. For example, several publications reported a negative correlation between the CO₂ release rate and seed viability and vigor, as observed in cabbage seeds (Kodde et al., 2012), wheat seeds (Dode et al., 2016), ryegrass seeds (Cavalcante et al., 2017) and soybean seeds (Cavalcante et al., 2023).

It could be that the negative correlation between the respiration rate and seed viability and vigor arose from oxidative stress induced by ethanol exposure, since ethanol exposure can be toxic to seeds, attacking cellular structures and inducing oxidative stress. It has been reported that ethanol interacts with the lipid bilayer of cell membranes, causing structural alterations such as increased membrane fluidity and permeability (Toth et al., 2014). This heightened fluidity and permeability can lead to increased electrolyte leakage; consequently, the cell membrane loses its ability to regulate the influx and efflux of substances. In the current study, cell

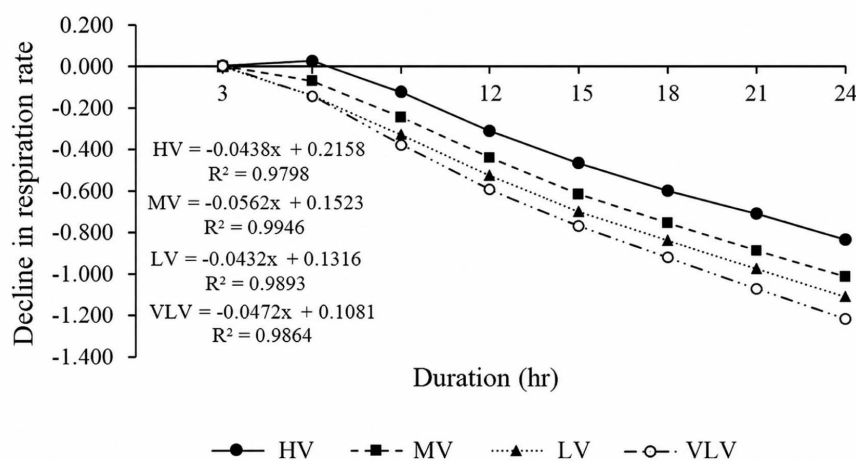
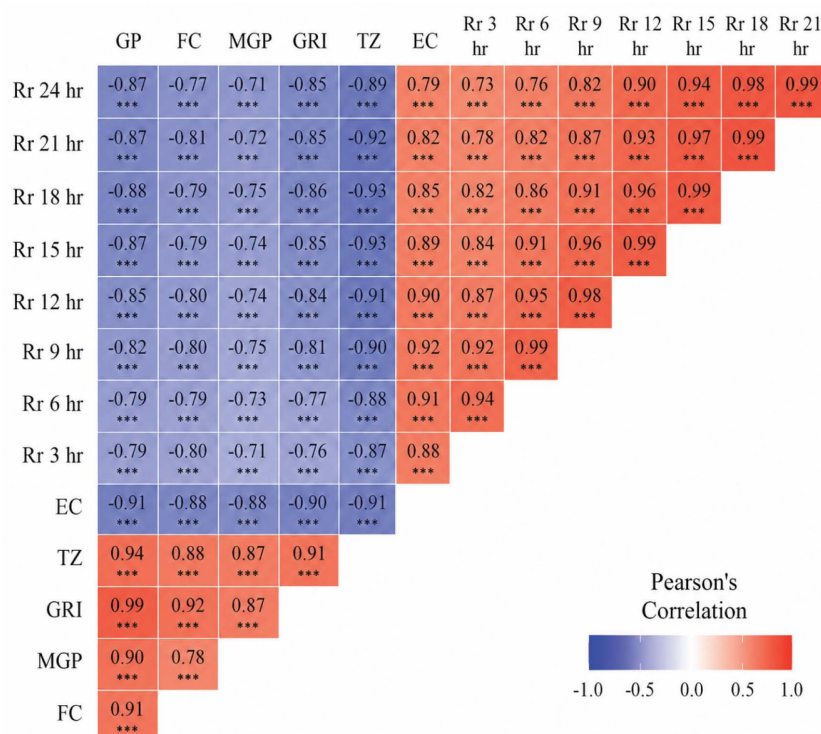


Fig. 4 Decline in respiration rate of groundnut seed lots with high vigor (HV), medium vigor (MV), low vigor (LV) and very low vigor lot (VLV). R² = coefficient of determination and dotted lines indicate linear regression equations.



ns $p \geq 0.05$; * $p < 0.05$; ** $p < 0.01$; and *** $p < 0.001$

Fig. 5 Pearson's correlation coefficient between groundnut seed physiological quality and real-time respiration rate. GP = germination percentage; FC = first count germination; MGP = maximum germination percentage; GRI = germination rate index; TZ = viability test by tetrazolium; EC = electrical conductivity; Rr 3–24 hr = respiration rate at duration of 3–24 hours, respectively. ns = not significant.

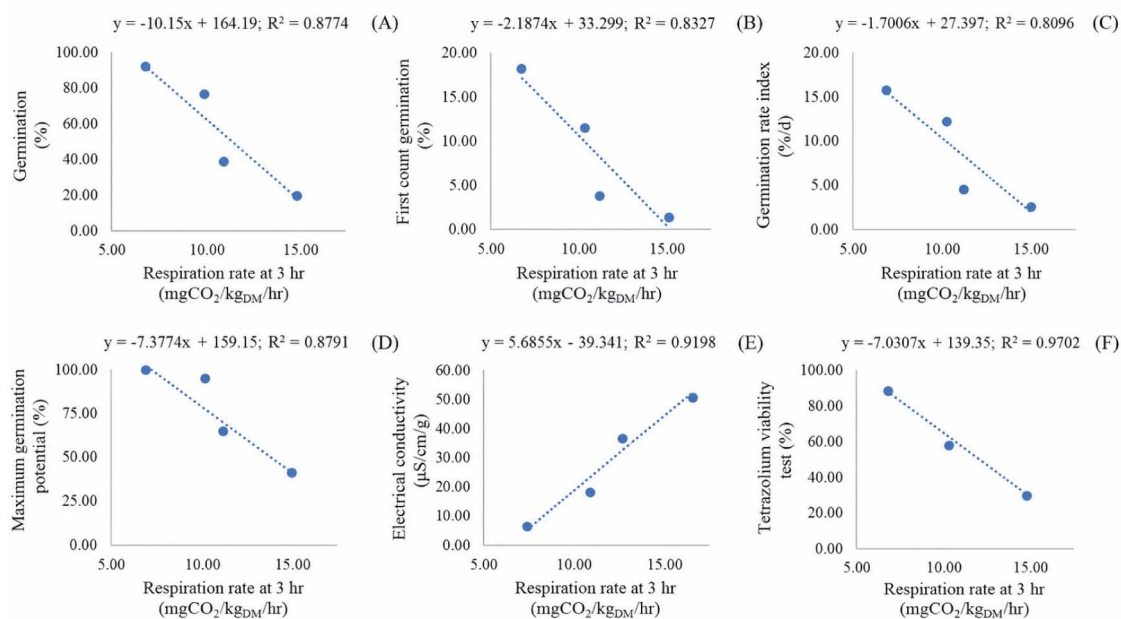


Fig. 6 Regression of groundnut seed physiological quality variables on the 3 hr real-time respiration rate. (A) Germination, (B) First count germination, (C) Germination rate index, (D) Maximum germination potential, (E) Electrical conductivity, and (F) Tetrazolium viability test. Dotted lines represent the regression equations. DM = dry mass.

membrane damage was indicated by the EC values, as the seed exposed to ethanol had high EC values, signifying increase membrane leakage.

This oxidative stress triggered the activation of a defence mechanism in the form of uncoupling respiration involving uncoupling proteins and alternative oxidase (Barreto et al., 2020; Popov et al., 2021). The activation of this pathway is aimed primarily at mitigating further ROS production, although this process causes electrons to flow without accompanying ATP synthesis. Consequently, the respiratory activity increased rapidly as the cells attempted to compensate for the loss of energy efficiency (Tiwari et al., 2002; Begcy et al., 2011). However, due to the cell membrane damage from ethanol exposure, electrons from the NADH dehydrogenase and the ubiquinone cycle continued to leak and interacted with oxygen to form massive amounts of ROS. This increase in efficient respiration, combined with the accumulation of toxic ethanol compounds, accelerated the depletion of energy reserves and caused permanent membrane damage, ultimately leading to the loss of seed viability and vigor (Woodstock and Taylorson, 1981; Dumont and Rivoal, 2019).

In conclusion, the current study demonstrated that ethanol vapor ageing in vacuum containers was effective at generating seed lots with varying quality levels. The real-time respiration rate based on CO₂ release showed potential as a rapid and non-destructive indicator of seed quality. However, this study was limited to a single groundnut variety and a small number of seed lots and utilized ethanol-induced deterioration rather than natural ageing during storage.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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