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Respiration Dynamics and a Rapid Colorimetric Assay for Seed Vigour Assessment in Paddy and Lentil

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ABSTRACT

The experiment was conducted during October, 2024–June, 2025 at Indian Agricultural Research Institute, New Delhi to study seed respiration and vigour assessment in lentil and paddy. Seed respiration was one of the earliest metabolic activities recorded in the imbibed seeds. Seed ageing impairs cellular function, leading to reduced respiration. This study evaluated CO_2 evolution in three seed lots, each of paddy and lentil, using an infrared CO_2 sensor. Lentil exhibited the highest CO_2 evolution (854.93 ppm seed^{-1}), while paddy recorded the lowest CO_2 evolution (429.65 ppm seed^{-1}). Significant within-crop variation was observed. In lentil, the lot L-4727(23-24), which had the highest germination, also recorded the highest CO_2 release (1034.73 ppm seed^{-1}), whereas L-4727(19-20) showed the lowest (707.87 ppm seed^{-1}). Similarly, in paddy, PB 1847 recorded the highest CO_2 evolution (446.69 ppm seed^{-1}) and PB 1985 recorded the lowest (418.77 ppm seed^{-1}). A rapid colorimetric vigour test was developed by trapping the CO_2 in the bromothymol blue (BTB) solution. OD_{430} values showed strong correlations with vigour index-I (R^2=0.96) and vigour index-II (R^2=0.93) in paddy and with vigour index-I (R^2= 0.98) and vigour index-II (R^2=0.76) in lentil. High-vigour seeds consistently exhibited higher respiration rates and higher OD_{430} values, validating BTB as a reliable indicator for vigour assessment. The present study presented a novel respiration-based rapid colorimetric vigour test capable of distinguishing seed lot vigour within 7 h.

KEYWORDS: Bromothymol blue, lentil, optical density, paddy, seed respiration

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1. INTRODUCTION

Respiration is a one of the earliest metabolic processes that occur after imbibition, resulting in the consumption of oxygen and the generation of carbon dioxide (Valle et al., 2021). It occurs in the mitochondria, where cells generate energy (Logan, 2006; Ponce et al., 2013). Respiration in mature dry seeds is usually very low when compared to germinating seed or developing seeds (Valle et al., 2021, Wang et al., 2018). Seed respiration is a vital physiological process that plays a key role in producing the metabolic energy needed for biological processes and various seed function (Bewley et al., 2012; Ren et al., 2023). Seed respiration exhibits distinct phases during and after imbibition, with an initial rapid increase in respiration as metabolic activity resumes (Bewley et al., 2012; Carillo-Reche et al., 2021). Vigorous seeds exhibit a higher respiration rate, which leads to increased ATP production, essential for germination and seedling growth. In contrast, aged or poor-quality seeds display lower and irregular respiration patterns, which are associated with poor germination and reduced seed vigour (Huang et al., 2020). Studying seed respiration is therefore essential for understanding the physiological and biochemical mechanisms underlying seed vigour, metabolism, and germination (Mendes et al., 2009; Bradford et al., 2013; Powell A.A. 2017). Seed respiration has been assessed using various methods, including the small basket method, the Warburg microrespirometer, Pettenkofer method, Q2 oxygen sensor technology, gas chromatography, and non-dispersive infrared (NDIR) CO_2 sensors (Gao et al., 2023). The Q2 instrument, known as the Seed Respiration Analyzer, developed by ASTEC Global, measures the oxygen consumption rates of individual seeds continuously during imbibition and germination (Bradford et al., 2013; Powell, 2017; Bello et al., 2016). Seed Respiration Analyser record the rate of oxygen consumption during the seed imbibition by generating the ASTEC values. Based on these ASTEC values, Q2 system provides a complete overview of seed vigour (Powell, 2017). The rate of respiration can also be measured by the amount of CO_2 evolved. Using Pettenkofer method, CO_2 evolution served as an indicator for the assessment of vigour in soybean (Dode et al., 2013); okra (De sousa et al., 2018), Similarly, CO_2 evolution was also used as a method to identify vigour of seed lots of West Indian gherkins (Leite et al., 2019). The seed respiration by CO_2 can also be measure by using acid-base indicators. The use of acid-base indicators as a proxy for measuring CO_2 evolution has been previously utilized for measuring soil respiration (Silva et al., 2016), microbial growth (Noshahri et al., 2023). The acid-base indicators are substances that change colour according to the pH of solutions. Bromothymol Blue (BTB) is a pH indicator commonly used in laboratory to detect pH changes visually from 6 to 7.6 pH. In acidic conditions (pH<6.0), BTB exists in its protonated form (HIn), appearing yellow and showing an absorption maximum around 430-nm. In basic conditions (pH>7.6), it transforms into its deprotonated form (In^-), which is blue and has an absorption peak near 615 nm. Its pH transition range of 6.0 to 7.6 makes it especially suitable for identifying slight variations near neutral pH (Skoog et al., 2016). Seed respiration has been previously reported as an effective indicator for seed vigour, but its measurement requires sophisticated and expensive instruments such as Q2 system. Therefore, there is a need for a cost-effective, rapid and reliable method to quantify seed respiration for vigour assessment. The present study aimed to develop a respiration-based vigour test that detected CO_2 evolution from pre-imbibed seeds and provided vigour estimates within 7 h or less. This rapid assay offered a valuable alternative to conventional vigour tests, which required several days to complete.

2. MATERIALS AND METHODS

2.1. Study area

The experiment was conducted during October, 2024–June, 2025 at Indian Agricultural Research Institute, New Delhi to study respiration and vigour assessment in lentil and paddy seeds.

2.2. Seed materials

Seed lots of paddy (*Oryza sativa* L.)-PB 1847, PB 1718 and PB 1985 and lentil (*Lens culinaris* L.)-L-4727(23–24), KBL-104(19–20) and L-4727(19–20) were obtained from the Division of Genetics, ICAR-Indian Agricultural Research Institute (IARI), New Delhi.

2.3. Germination and vigour assessment of lentil and paddy seed lots

The germination test for paddy and lentil was conducted by using the between paper method by using seeds (n=50) in triplicate (Anonymous, 2025) with some modification. Germination counts were recorded at the final count, and the total germination percentage (GMAX, %) and total normal seedlings (TNS, %) were determined according to the ISTA Handbook on Seedling Evaluation (Anonymous, 2018). From each replication, ten normal seedlings were randomly selected for measurement of seedling length. These seedlings were subsequently dried in a hot-air oven at 70°C for 48 h to obtain seedling dry weight. Vigour indexes I and II were calculated according to Abdul-Baki and Anderson (Abul baki and Anderson 1973).

2.4. Measurement of CO_2 evolution using an infrared sensor

To quantify differential CO_2 evolution in seeds with varying viability, paddy (n=10), and lentil (n=3) seeds where were pre-soaked for 3 h. The seeds were placed in 50 ml

centrifuge tube (Tarsons, India Pvt. Ltd) equipped with an infrared CO_2 sensor (Model MH-Z19B, Zhengzhou Winsen Electronics Technology Co., Ltd., People's Republic of China). An Arduino Nano was used a microcontroller. CO_2 release was monitored for 4 h, with measurements recorded at 30 min intervals, and the rate of CO_2 evolution was expressed as the mean concentration in parts million^{-1} (ppm seed^{-1}).

2.5. Vigour assessment by colorimetric method

Half a grams of seeds paddy (~20 seeds) or lentil (~18 seeds) were pre-soaked in distilled water for 3 h. The soaked seeds were placed in a seed holder and transferred into a 15 ml airtight incubation tube (Borosil India Pvt. Ltd.) containing 4 ml of BTB indicator solution, ensuring that the seeds did not come in direct contact with the solution (Figure 1). The incubation tube containing seeds were kept in an incubator set at 25±1°C. BTB was procured from Sisco Research Laboratories Pvt. Ltd. India. BTB indicator solution was prepared by dissolving 10 mg BTB in 100 ml of distilled water, and the pH was adjusted to 9.5 using 0.1 N NaOH. For each seed lot, nine incubation bottles were prepared to record optical density at 30-min intervals over a 4-h period (0, 30, 60, 90, 120, 150, 180, 210 and 240 min) in three replications.

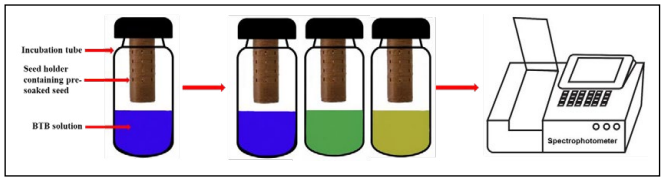

Figure 1: Procedure for assessing seed vigour using bromothymol blue (BTB). Seeds are first soaked in water for 3 h, followed by the addition of 4 ml of BTB solution (pH 9.5) into a 15 ml vials. A seed holder is placed inside to secure the seed, and the tube is sealed airtight and incubated for 4 h. During incubation, optical density at 430 nm and 615 nm has been measured at 30 min time interval by using the spectrophotometer and pH of the solution were also recorded at the same time

After incubation for different period, the OD of the BTB solution was measured using spectrophotometer (UV-1800 UV-VIS spectrophotometer, Shimadzu, Japan). OD values were measured at 430 nm and 615 nm, corresponding to the absorbance peaks of BTB. At the same time the pH of the BTB solution was recorded at 30 min interval up to 240 min. The OD values obtained were correlated with the vigour indices (Abdul-Baki and Anderson, 1973).

2.6. Electrical conductivity measurement

The electrical conductivity of paddy and lentil seeds was measured according to ISTA rules (Anonymous, 2025) with minor modifications. Fifty seeds in triplicates were soaked in 50 ml of distilled water for 24 h. The conductivity reading was recorded by using the conductivity meter (Eutech PC 700, Thermo Fisher Scientific, USA). The EC was expressed as μS cm^{-1} g^{-1} using the formula:

$$\text{Conductivity } (\mu S\ cm^{-1}\ g^{-1}) = \frac{\text{Conductivity reading } (\mu S\ cm^{-1}) - \text{Background reading}}{\text{Weight of the replicate (g)}}$$

2.7. Dehydrogenase activity

The dehydrogenase activity was measured as per Kittock and Law, 1968 with some modifications. Twenty seeds were soaked in distilled water for 18 h. After soaking, the seed coat of lentil was removed, while in paddy a 3/4th longitudinal cut was made through the embryo and endosperm. The seeds were then immersed in a freshly prepared 1 % solution of 2,3,5-triphenyl tetrazolium chloride (TZ). Lentil seeds were incubated in TZ for 6 h and paddy seeds for 2 h at 30°C in the dark. After the staining, the seeds were transferred to test tube containing the 10 ml of methyl cellosolve and kept in darkness overnight. The OD values was measured at 470 nm using spectrophotometer (UV-1800 UV-VIS spectrophotometer, Shimadzu, Japan), and values were expressed as OD gram seed^{-1}.

2.8. Statistical analysis

Data collected for all parameters were subjected to statistical analysis using RStudio (version 10) and SAS software (version 9.4). The Least Significant Difference (LSD) values were computed for mean comparison. Contrast analysis using matrix functions in RStudio was performed instead of a multifactorial CRD analysis, and variance ratios were tested for significance at the p=0.05% probability level. Repeated-measures ANOVA was carried out using the General Linear Model (PROC GLM) procedure in SAS (version 9.4). Percentage germination data were transformed to arcsine values prior to analysis.

3. RESULTS AND DISCUSSION

3.1. Seed respiration in paddy and lentil seed lots

3.1.1. Seed respiration in paddy seed lots

The respiration rate of three paddy seed lots-PB 1847, PB 1718, and PB 1985, was measured over 4 h period at 30 m intervals using an infrared CO_2 sensor was presented in (Figure 2). The corresponding germination percentages of these varieties were 95%, 72%, and 47%, respectively (Table 1). The line graph of CO_2 evolution over time (figure 2) showed a progressive increase in CO_2 release across all varieties. Among them, PB 1847 exhibited the highest respiration rate (446.69 ppm seed^{-1}), followed by PB 1718 (423.46 ppm seed^{-1}), while PB 1985 recorded the lowest value (418.77 ppm seed^{-1}). These trends indicated high-germination seed lots showed a rapid rise in respiration, whereas low-germination seed lots exhibited only a modest and gradual increase.

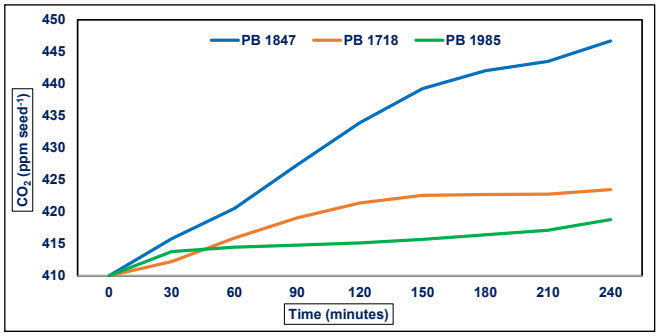

Figure 2: Line graph showing CO_2 evolution in seeds of three paddy varieties (PB 1847, PB 1718, and PB 1985). CO_2evolution was recorded using an infrared CO_2 sensor over a 4 h period at 30 min intervals. Values represent the mean CO_2 concentration expressed as ppm seed^{-1}

3.1.2. Seed respiration in lentil seed lots

The respiration rate of three lentil varieties-L-4727(23-24), KBL-104(19-20), L-4727(19-20), was measured over 4 h at 30 m intervals using an infrared CO_2 sensor was presented in (figure 3). The corresponding germination percentages of these varieties were 99%, 76%, and 50%, respectively (Table 3). The line graph of CO_2 evolution over time (figure 3) showed a progressive increase in CO_2 release across all varieties. Among them, L-4727(23-24) exhibited the highest respiration rate (1034.73 ppm seed^{-1}), followed by

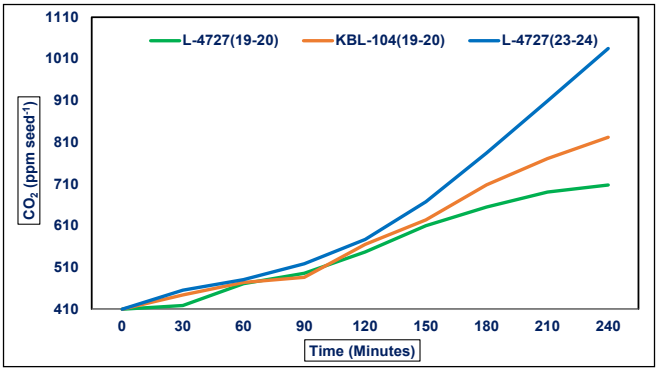

Figure 3: Line graph showing CO_2 evolution in seeds of three lentil varieties (L-4727(23–24), KBL-104(19–20), and L-4727(19–20)). CO_2 evolution was recorded using an infrared CO_2 sensor over a 4 h period at 30 min intervals. Values represent the mean CO_2 concentration expressed as ppm seed^{-1}

Table 1: Comparison of germination, seedling vigour, electrical conductivity, and dehydrogenase activity in paddy varieties

Varieties	Total G %	Percent germination	Vigour index I	Vigour index II	EC (µS cm^{-1} g^{-1})	Dehydrogenase activity (OD g^{-1})
PB 1847	95 (77.52)^{a*}	92 (73.57)^{a}	3201.09^{a}	9.70^{a}	38.80^{a}	0.33^{a}
PB 1718	72 (58.05)^{b}	64 (53.13)^{b}	2146.82^{b}	6.75^{b}	45.36^{b}	0.15^{b}
PB 1985	47 (43.08)^{c}	33 (35.26)^{c}	1151.81^{c}	4.46^{c}	56.10^{c}	0.05^{c}
CD (p=0.05)	3.99	5.61	332.01	1.06	5.98	0.05

*Values in the parenthesis are arcsine-transformed values. Total G (%) includes all germinated seed. Percent germination is based on Anonymous, 2025 criteria for seedling evaluation. Different letters denote significant differences among varieties (p<0.05). CD: Critical difference

KBL-104(19-20) (822.19 ppm seed^{-1}), while L-4727(19-20) recorded the lowest value (707.87 ppm seed^{-1}). These trends indicated germination seed lots showed a rapid rise in respiration, whereas low-germination seed lots exhibited only a modest and gradual increase.

Repeated-measures ANOVA demonstrated a significant effect of variety on CO_2 evolution in both paddy (Table 2; p=0.04) and lentil (Table 4; p=0.02), indicating clear differences in respiration among the varieties of each crop. Time exerted a highly significant influence on CO_2 evolution in both cases (p<0.0001), confirming substantial changes over the incubation period. Furthermore, significant time×variety interactions for paddy (p=0.0008) and lentil (p<0.0001) revealed that the rate and temporal pattern of CO_2 evolution varied among varieties over time.

Table 2: Analysis of variance (ANOVA) showing the p-values for the effects of variety, time, and their interaction (time×variety) on CO_2 evolution, optical density at 430 nm (OD_{430}) and 615 nm (OD_{615}), and pH in paddy

Source of variation	p-value			
	CO_2	OD_{430}	OD_{615}	pH
Variety	0.04*	0.01**	0.01*	0.01*
Time	<.0001***	<.0001***	<.0001***	<.0001***
Time×Variety	0.0008**	<.0001***	<.0001***	<.0001***

*: p<0.05, **: p<0.01, and ***: p<0.001

In the present experiment, was conducted to assess the respiration rate in two crops i.e., paddy and lentil using three seed lots of each crop with varying vigour levels, with

Table 3: Comparison of germination, seedling vigour, electrical conductivity, and dehydrogenase activity in lentil varieties

Varieties	Total G %	Percent germination	Vigour index I	Vigour index II	EC ($\mu S\ cm^{-1}\ g^{-1}$)	Dehydrogenase activity ($OD\ g^{-1}$)
L-4727 (23–24)	99 (83.35)[a*]	91 (72.20)[a]	2395.69[a]	13.37[a]	159.67[a]	2.21[a]
KBL-104 (19–20)	76 (60.44)[b]	55 (48.06)[b]	1845.42[b]	8.43[b]	239.78[b]	1.75[b]
L-4727 (19–20)	50 (45)[c]	40 (39.81)[c]	1385.07[c]	7.85[b]	309.68[c]	1.48[c]
CD= (p<0.05)	5.02	10.48	295.76	1.03	9.61	0.12

*Values in the parenthesis are arcsine-transformed values. Total G (%) includes all germinated seed. Percent germination is based on Anonymous, 2025 criteria for seedling evaluation. Different letters denote significant differences among varieties (p<0.05). CD: critical difference

Table 4: Analysis of variance (ANOVA) showing the p-values for the effects of variety, time, and their interaction (time×variety) on CO_2 evolution, optical density at 430 nm (OD_{430}) and 615 nm (OD_{615}), and pH in lentil

Source of variation	p-value			
	CO_2	OD_{430}	OD_{615}	pH
Variety	0.02*	0.0002**	0.0002**	0.004**
Time	<.0001***	<.0001***	<.0001***	<.0001***
Time×Variety	<.0001***	<.0001***	<.0001***	<.0001***

*: p<0.05; **: p<0.01; ***: p<0.001

germination percentages ranging from 99% to 47%. At the end of 4 h incubation period, the CO_2 evolved $seed^{-1}$ was 429.65 ppm in paddy and 854.93 ppm in lentil. The CO_2 evolution in lentil was nearly twice than that of paddy (Appendix table 1). The respiration rate in monocot (paddy) and dicot (lentil) could differ. In seeds, not all tissues were alive, the living tissues included embryo, cotyledon and aleurone layer. The starch and other reserve portion of the seeds comprised of the non-living tissue (Boesewinkel and Bouman, 1984). When seeds of comparable size or mass were evaluated, dicot seeds generally exhibited higher respiration rates because they contained a greater proportion of living tissue, particularly the cotyledons, which made up most of their seed structure (Ibl and Stroger, 2014; Ariza-Nieto et al., 2007; Sreenivasulu and Wobus, 2013) V. Mitochondria were commonly called the powerhouse of the cell; thus, CO_2 evolution took place in the mitochondria of the active living cell (Ratajczak et al., 2019).

3.2. Vigour estimation by BTB indicator in paddy and lentil

3.2.1. Vigour estimation by BTB indicator in paddy

Significant varietal differences were observed in vigour indices. PB 1847 recorded the highest vigour index I (3201.09), followed by PB 1718 (2146.82), whereas the lowest value was recorded in PB 1985 (1151.81). Similarly,

Appendix Table 1: Mean CO_2 evolution of two crops and ANOVA results for the effects of crop, time, and crop×time interaction

Time (min)	Crop		Average
	Paddy	Lentil	
0	410.00	410.00	410.00h
30	413.93	439.59	426.76gh
60	416.96	475.28	446.12fg
90	420.39	500.51	460.45f
120	423.48	563.27	493.37e
150	425.84	633.83	529.83d
180	427.04	715.86	571.45c
210	427.78	790.21	608.99b
240	429.65	854.93	642.29a
Average	421.67a	598.16b	
ANOVA	F value	p value	
Factor (time)	154.458	<0.0001	
Factor (crop)	1568.519	<0.0001	
Interaction (time× crop)	131.890	<0.0001	

Means followed by different letters within a column or row indicate significant differences (p<0.05)

vigour index II also varied significantly among varieties, where highest value was recorded in PB 1847 (9.70), followed by PB 1718 (6.75) and lowest in PB 1985 (4.46) (table 1).

3.2.1.1. OD at 430 nm

At 430 nm, OD increased progressively overtime in all varieties, due to acidification caused by seed respiration (figure 4A). Among the varieties, PB 1847 showed the highest OD values at all time points, reached a maximum of 1.39 at 240 min followed by PB 1718 (1.23) at 240 min, while lowest OD was recorded in PB 1985 (1.12). The

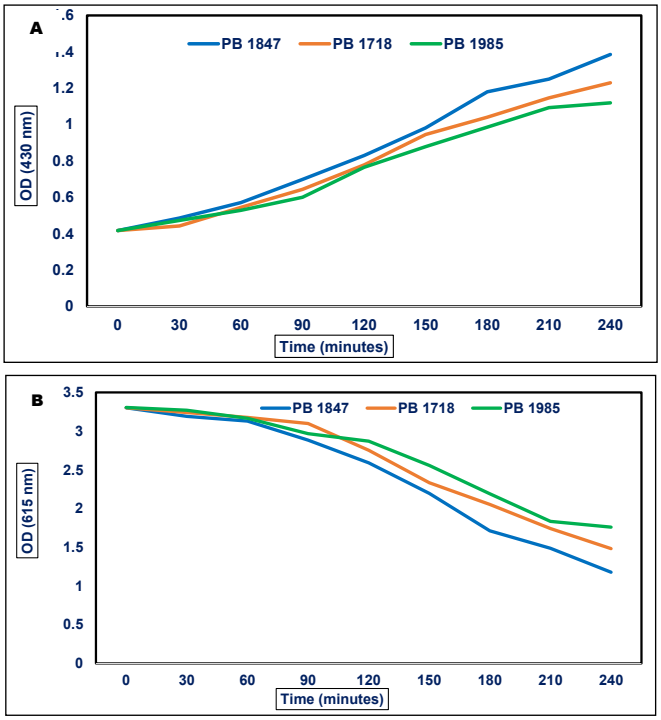

Figure 4: Optical density (OD) of three paddy varieties namely PB 1847, PB 1718 and PB 1985 recorded over 240 min: (A) OD at 430 nm showing a progressive increase with time, and (B) OD at 615 nm showing a steady decline as time progresses

difference in OD increased between varieties was most pronounced in PB 1847.

3.2.1.2. OD at 615 nm

At 615 nm, OD decreased progressively in overtime in all varieties, due to acidification caused by seed respiration (figure 4B). Among the varieties, PB 1847 displayed the steepest decline, with OD decreased from 3.30 at 0 min to 1.18 at 240 min, followed by PB 1718 (1.48) at 240 min, while PB 1985 recorded lowest decline on OD values from initial 3.31 to 1.76 by 240 min. The difference among the varieties became clearer after 120 min. The difference in OD decreased between varieties was most pronounced in PB 1847 (Figure 5a and b).

3.2.1.3. Change in pH of BTB indicator in paddy

pH measurement of the BTB solution at 30 min interval showed a significant decline over the 4 h incubation period across the three paddy varieties, indicated progressive acidification of BTB solution due to seed respiration (figure 6). The highest decline in pH was observed in PB 1847, where pH decreased from initial 9.50 to 6.85, PB 1718 exhibited a moderate decline from 9.50 to 7.29, while PB 1985 showed the least change, with pH decreasing from 9.50 to 7.51. The corresponding colour transition of the BTB solution, indicating the acidification, was presented in figure 7.

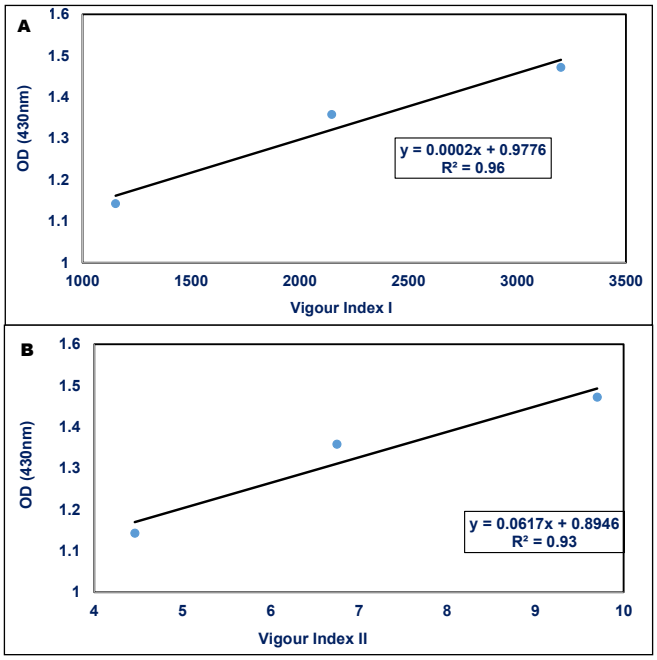

Figure 5: Linear regression between the optical density (OD) values (measured at 430 nm) of bromothymol blue solution after incubation with seeds from three paddy seed lots-PB 1847, PB 1718 and PB 1985 and their (A) vigour index-I and (B) vigour index-II. Each data point represents the mean of three replicates (n=3)

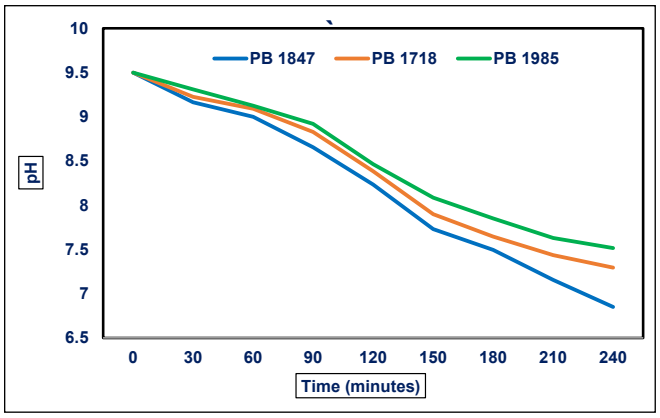

Figure 6: Decrease in pH of the BTB solution over the time from 0 to 240 min in three paddy varieties. Each data point represents the mean of three replicates (n=3)

Repeated-measures ANOVA (Table 2) indicated a significant effect of variety on optical density (OD) at 615 nm and 430 nm (p=0.01) as well as on pH (p=0.004). Time exerted a highly significant influence on both OD and pH values (p<0.0001). Significant time× variety interactions for OD and pH (p<0.0001) demonstrated that the temporal patterns of change differed among the varieties.

3.2.2. Vigour estimation by BTB indicator in lentil

Significant varietal differences were observed in vigour indices (table 3). L-4727(23–24) recorded the highest

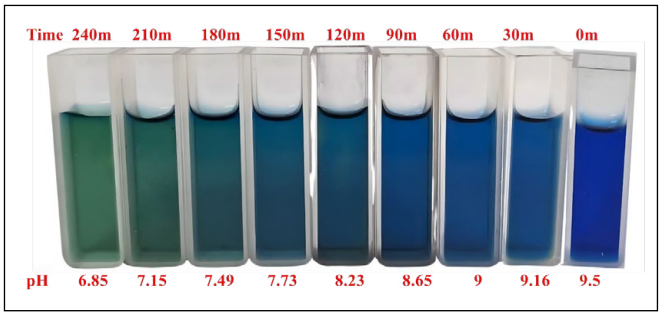

Figure 7: Representative colour change pattern of bromothymol blue of pre-soaked seeds of paddy variety PB 1847 at different time intervals

vigour index I (2395.69), followed by KBL-104(19–20) (1845.42), whereas the lowest value in L-4727(19–20) (1385.07). Similarly, vigour index II also varied significantly among varieties, where highest value in L-4727(23–24) (13.37), followed by KBL-104(19–20) (8.43) and lowest in L-4727(19–20) (7.85).

3.2.2.1. OD at 430 nm

At 430 nm, OD increased progressively over time in all varieties, due to acidification caused by seed respiration (figure 8A). L-4727(23–24) recorded highest OD value of 1.89 at 240 min, followed by KBL-104(19–20) (1.65), while lowest OD was recorded in L-4727(19–20) (1.59). The difference in OD increase between varieties was most pronounced in L-4727(23–24).

3.2.2.2. OD at 615 nm

At 615 nm, OD decreased progressively in overtime in all varieties, due to acidification caused by seed respiration (figure 8B). Among the varieties, L-4727(23–24), displayed the steepest decline, with OD decreasing from 3.32 to 0.27, followed by KBL-104(19–20) at 240 min, while L-4727(19–20) recorded lowest declined on OD values from initial, 3.30 to 0.66 by 240 min. respectively. The difference in OD decrease between varieties was most pronounced in L-4727(23–24).

3.2.2.3. Change in pH of BTB indicator in lentil

pH measurement of the BTB solution at 30 min interval showed a significant decline over the 4 h incubation period across the three lentil varieties, indicaticated progressive acidification of BTB solution due to seed respiration. The greatest pH reduction occurred in L-4727(23–24), where pH decreased from 9.50 to 5.42. KBL-104(19–20) showed the next highest declined, from 9.50 to 5.71, while L-4727 (19–20) exhibited the lowest pH decline change, with pH decreasing from 9.50 to 5.93 (figure 10). The corresponding colour transition of the BTB solution, indicating the acidification, was presented in figure 11.

Repeated-measures ANOVA (Table 4) revealed a significant effect of lentil variety on pH (p =0.01) and on optical

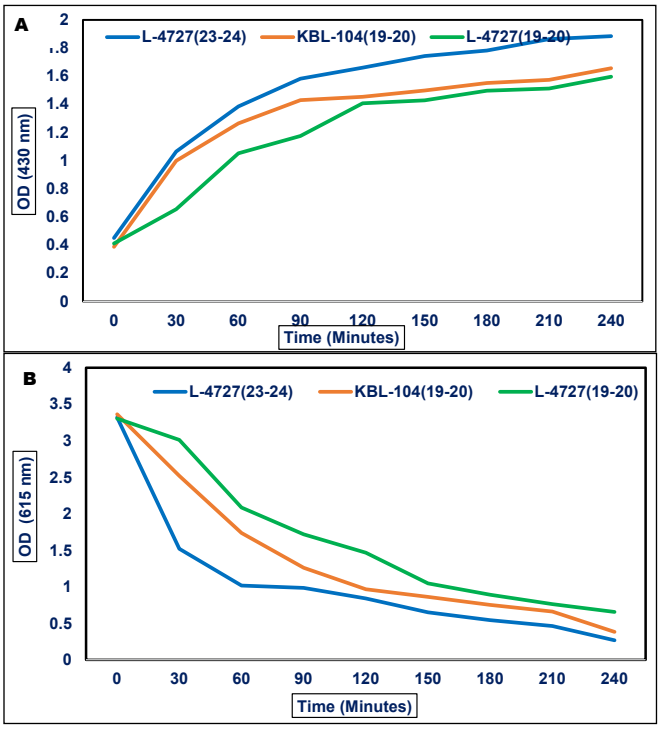

Figure 8: Optical density (OD) of three lentil varieties namely L-4727(23–24), KBL-104(19–20) and L-4727(19–20) recorded over 240 min: (A) OD at 430 nm showing a progressive increase with time, and (B) OD at 615 nm showing a steady decline as time progresses

density (OD) measured at 615 nm and 430 nm (p=0.0002), indicated clear differences among the varieties. Time had a highly significant influence on both pH and OD values (p<0.0001). Furthermore, significant time×variety interactions for pH and OD (p<0.0001) demonstrated that the temporal patterns of pH and OD changes varied among the lentil varieties over time.

Seed vigour was an important seed quality attribute which ensured rapid, uniform germination and healthy seedling growth, even under biotic stress. Traditional vigour tests like first count, filed emergence, vigour indices were time-consuming. One of the major objectives of the present study was to develop a rapid vigour test based on seed respiration, which did not required any sophisticated instruments and could be easily done using the common lab instruments. The seed respiration was an indicator of vital cellular activity and has been associated with seed vigour (Zhao et al., 2009; Zhao and Zhang, 2012). The three seed lots each in paddy and lentil were harvest in different years and naturally aged for (1–3 years) which had different vigour level, measured as vigour indices (Abul baki and Anderson, 1973) (table 1, 3).

There was also a clear difference in the rate of CO_2 evolution among the seed lots of paddy and lentil after 4 h incubation period (figure 2, 3). In paddy, the highest CO_2 evolution (446.69 ppm seed^{-1}) was recorded variety, PB 1847 (figure

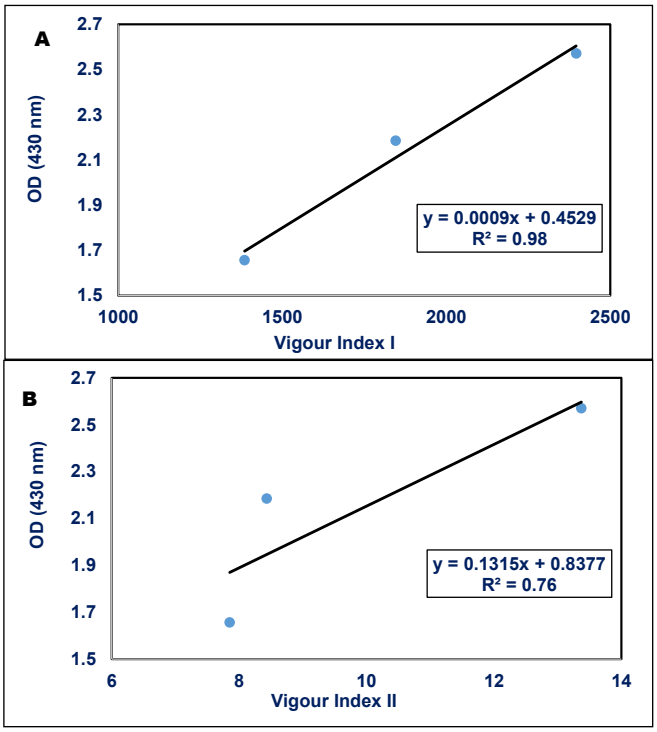

Figure 9: Linear regression between the optical density (OD) values (measured at 430 nm) of bromothymol blue solution after incubation with seeds from three paddy seed lots- L-4727(23–24), KBL-104(19–20) and L-4727(19–20) and their (A) vigour index-I and (B) vigour index-II. Each data point represents the mean of three replicates (n=3)

2), which also had highest germination (95%), vigour index I (3201.09) and vigour index II (9.70) (Table 1). In contrast, the lowest CO_2 evolution (418.77 ppm seed^{-1}) was observed in PB 1985 (figure 2), the lot with lowest germination (47%), vigour index I (1151.81,) and vigour index II (4.46) (Table 1). Similarly, in lentil, the highest CO_2 evolution (1034.73 ppm seed^{-1}) was recorded in variety L-4727(23–24), which had highest germination (99%), vigour index I (2395.69) and vigour index II (13.37), whereas lowest CO_2 evolution (707.87 ppm seed^{-1}) was recorded in L-4727(19–20), the seed lot with the lowest germination (50%), vigour index I (1385.07), and vigour index II (7.85) (Table 3, figure 3).

In paddy, strong relationships were observed between OD_{430} and vigour index I (R^2=0.96) and vigour index II (R^2=0.93). Similarly, in lentil, OD_{430} showed high coefficients of determination with vigour index I (R^2=0.98) and vigour index II (R^2=0.76) (figure 5, 9). These results demonstrated that the colorimetric response of the BTB indicator reliably reflected differences in seed vigour. Since higher-vigour seeds respired more intensely, they produced more CO_2, resulting in greater acidification and correspondingly larger OD changes (Naik et al., 2026). pH measurements taken at different time intervals confirmed consistent differences among varieties in both crops (figure 6, 10). This explained

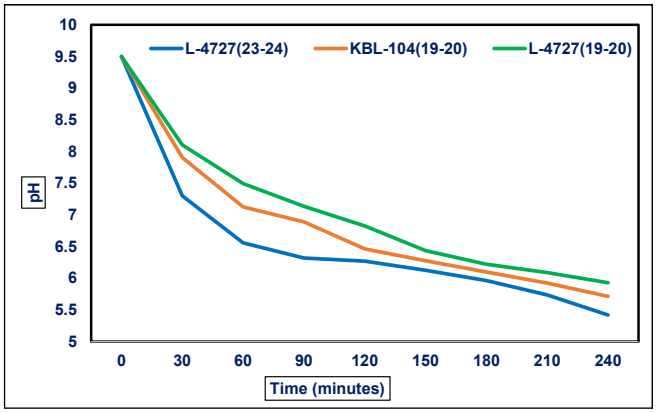

Figure 10: Decrease in pH of the BTB solution over the time from 0 to 240 min in three lentil varieties. Each data point represents the mean of three replicates (n=3)

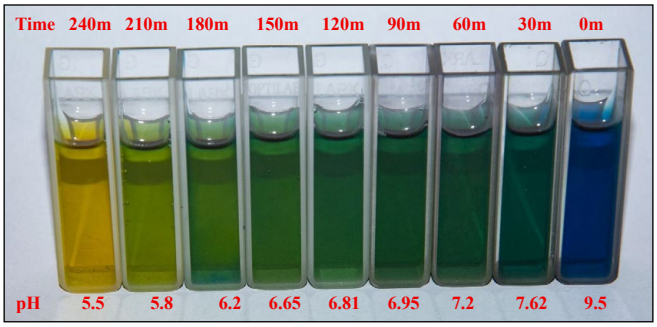

Figure 11: Representative colour change pattern of bromothymol blue of pre-soaked seeds of lentil variety L-4727(2023–24) at different intervals

the clear separation in OD values among varieties (figures 4, 8). These findings aligned with earlier work (Zhao et al., 2009), who reported significant correlations with seed respiration parameters like oxygen metabolism rate and critical oxygen pressure and seed vigour in different lots of maize. CO_2 released by seeds have also been previously reported as reliable indicator for vigour evaluation in soybean (Dode et al., 2013) and okra (De sousa et al., 2018).

3.3. Electric conductivity of paddy and lentil seed lots

Significant different in the electric conductivity was observed among the paddy seed lots (Table 1). The variety PB 1847 which exhibited the highest vigour and germination, recorded the lowest EC value of (38.80 µS cm^{-1} g^{-1}), followed by the PB 1718 with 45.36 µS cm^{-1} g^{-1}. The highest EC value was observed in variety PB 1985 (56.10 µS cm^{-1} g^{-1}), which also had the lowest vigour and germination. Similarly, in lentil (Table 3), the variety L-4727(2023–24), which had the highest vigour and germination, recorded the lowest EC value (159.67 µS cm^{-1} g^{-1}). This was followed by KBL-104(2019–20) with 239.78 µS cm^{-1} g^{-1}, while the highest EC value was recorded in L-4727(2019–20) (309.68 µS cm^{-1} g^{-1}), which also showed the lowest vigour and germination.

3.4. Dehydrogenase activity in paddy and lentil seed lots

Dehydrogenase activity varied with the seed quality in both paddy and lentil (Table 1, 3). The paddy variety PB 1847 showed the highest value of 0.33 g^{-1}, followed by PB 1718 with OD value of 0.15 g^{-1}, while the lowest OD was recorded PB 1985 at 0.05 g^{-1}, indicating a significant difference among the seed lots. Similarly, dehydrogenase activity in lentil also varied with germination and vigour. The variety L-4727(2023–24) recorded the highest value of 2.21 g^{-1}, followed by KBL-104(2019–20) with 1.75 g^{-1}, while the lowest value was observed in L-4727(2019–20) at 1.48 g^{-1}.

Seed deterioration was an inevitable and a cumulative process. The loss of seed vigour and viability was closely associated with degradation of cellular membranes and impairment of antioxidant defence mechanisms (Ebone et al., 2019; Yalamalle et al., 2024). In this study lowest dehydrogenase activity was also observed in seed lots with low vigour and viability (Table 1, 3). Electrical conductivity, an indicator of membrane damage (Yalamalle et al., 2024; Parvar et al., 2025) was also higher in seeds with low vigour and viability (Table 1, 3). As the seed ages it led to reduced mitochondrial function, impaired energy metabolism and cellular respiration. As mitochondria were the primary sites of respiration (Ratajczak et al., 2019), this explained the reduced CO_2 evolution observed in the paddy and lentil seed lots with low vigour and viability.

4. CONCLUSION

Significant variation in CO_2 evolution was observed among the varieties in both crops, allowing clear differentiation based on vigour levels. The CO_2 released during respiration was captured in BTB solution, with absorbance measured at 430 nm and 615 nm at 30 min intervals up to 240 min. The optical density measurement had distinguished the varieties based on vigour status. Overall, the findings demonstrated seed respiration-based colorimetric as an novel approach for differentiating seed vigour in paddy and lentil.

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