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SpeedySeed viability kit: A rapid colourimetric assay for seed viability testing

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ABSTRACT

Seed viability and vigour are vital seed quality parameters determining the crop establishment. The traditional seed quality testing methods are time-consuming and require skilled personnel. Standard germination tests can take two to three weeks, and the tetrazolium test, though faster, demands technical expertise. The SpeedySeed Viability Kit™ offers a rapid, low-cost, on-farm alternative. The study was carried out during 2024–25 at ICAR-Indian Agricultural Research Institute, New Delhi to estimate the viability of seed lots of paddy (*Oryza sativa* L.) and lentil [*Lens culinaris* (L.) Medik.] by using SpeedySeed Viability kit. It works on the principle that viable, vigorous seeds release more CO_2 during respiration. This CO_2 is absorbed by a bromothymol blue indicator solution in a sealed tube, producing a colour change during the incubation—green/yellow for viable seeds and blue for non-viable seeds. The viability can be assessed in 6 h in paddy and 3 h in lentil, making it the fastest seed viability assessment method. The viability estimated closely matched the total viability by the standard germination test with a prediction accuracy ranging from 89–93% in paddy and 96–99% in lentil. The viability estimated by the kit was also validated by measuring the optical density value at 450 nm, where the lot with higher viability recorded the highest OD values. The linear regression with OD and their corresponding germination had a strong correlation with an R^2 value of 0.87 in paddy and 0.76 in lentil seed lots.

Keywords: Bromothymol blue, Colourimetric test, Respiration, Seed viability

Seed is the fundamental unit of agriculture and quality of seed is utmost important, as it directly affects crop establishment in the field and ultimately the yield (Yalamalle and Kuchlan 2016, Khalid *et al.* 2023, Kumar *et al.* 2023). Using high-quality seeds along with appropriate cultivation practices can substantially enhance crop yield by 15–20% (Chauhan *et al.* 2016).

Seed viability is a key parameter for assessing overall seed quality (Xia *et al.* 2019, Lamichaney *et al.* 2023, Pal *et al.* 2025) and it is usually measured by traditional methods like standard germination test, tetrazolium assay, electrical conductivity, X-ray radiography, and embryo excision (ISTA 2025). However, these methods being invasive, time consuming and labour intensive, require trained manpower (Rahman and Cho 2016, Rajabi *et al.* 2023).

Respiration is a vital physiological process, which begins immediately after imbibition, and continues until radicle emergence (Bewley *et al.* 2013), which reflects the metabolic status and closely associated with seed quality (Bradford *et al.* 2013, Guo *et al.* 2024). Seed respiration measurement through CO_2 or O_2 sensors, studied for evaluating the seed viability and vigour (Silva *et al.* 2021, Widajati *et al.* 2023).

Bromothymol blue (BTB) is an acid base indicator, exhibits distinct colour pattern depending on the *p*H of the solution, with yellow (*p*H <6), blue (*p*H> 7.6) and green colour (*p*H 6.0 –7.6) (Skoog *et al.* 2019, Yang *et al.* 2023). During the seed respiration, release CO_2, which reacts with water to form carbonic acid, thereby lowering the *p*H of the BTB solution that led to shift in the colour. Seed lots with higher germination have been reported previously to release more CO_2 (Dalzeli and Tomilson 2017, Widjati *et al.* 2023). The present study was carried out to develop a respiration-based rapid on-farm seed viability testing method using bromothymol blue in paddy (*Oryza sativa* L.) and lentil [*Lens culinaris* (L.) Medik.].

MATERIALS AND METHODS

Seed materials: The study was carried out during 2024–25 at ICAR-Indian Agricultural Research Institute (28°38′23″ N, 77°09′27″ E; at an elevation of 228.6 m amsl), New Delhi to estimate the viability of seed lots of paddy and lentil using SpeedySeed Viability kit. Five varieties of paddy namely PB 1121, PB 1509, PB 1692, PB 1886 and PB 1718, and five varieties of lentil i.e. L-7903, IPL-220, PL-4, LL-699 and K-75 were procured from the Seed Production Unit, ICAR-Indian Agricultural Research Institute, New Delhi.

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Standard germination test: The five seed lots of paddy and lentil were subjected to germination test by using seeds (n=50) in three replications through between paper method with some modification (ISTA 2025). The total germination percentage (G_{MAX}, %) and total normal seedlings (TNS, %) were determined according to the ISTA Handbook on Seedling Evaluation (2018) at final count, which is 14 days in paddy and 10 days in lentil.

Viability assessment by SpeedySeed viability kit: Colourimetric viability assessment was done by using bromothymol blue acid-base indicator, which was prepared at the concentration of 0.04 mg/mL. The *p*H of the indicator solution was adjusted to 9.5 and 8.5 for lentil and paddy, respectively using 0.1N NaOH. Standardisation of soaking duration and incubation duration for viability assessment using kit was made by soaking and incubating the seeds of variety PB 1121 of paddy having the actual germination percentage of 98%, and L-7903 of lentil with actual germination percentage of 99%, at different durations of 30, 60, 90, 120, 150, 180, 210 and 240 min. Very short duration that produced colourimetric results closely matches to the actual germination percentage of each crop was selected as appropriate soaking duration and incubation duration. The soaking and incubation durations were 4 h and 6 h for paddy, and 2.5 h and 3 h for lentil, respectively. Seed viability estimation using the kit involved soaking the seed for a pre-determined period, followed by incubation of a single pre-soaked seed in a 1.5 mL vial containing 250 µL of BTB solution (at the required *p*H). The seed was secured using a seed holder to prevent direct contact with the BTB solution, and the vial was tightly closed to ensure airtight conditions. At the end of incubation period (3 h in lentil and 6 h in paddy), the number of vials turning green or yellow, recorded as viable seed, and vials remain blue (initial colour) recorded as non-viable seed. 50 seeds with three replications of each variety have been tested calorimetrically by using the kit method (Fig. 1 and 2).

Measurement of optical density: At the end of incubation period i.e. 3 h or 6 h, viability assessed by visual observation was also validated by measuring the optical density (OD) of BTB solution at 450 nm spectrophotometrically, using a plate reader such as the BR Biochem Life Science Pvt. Ltd., India (Model: BR-580). After completion of the incubation period, 220 µL of the BTB solution was pipetted out from each tested vial, and the OD values were recorded. The OD values obtained were correlated with their corresponding germination percentage.

Statistical analysis: Viability estimation by the kit method was compared with the standard germination test using the GLM-PROC through SAS software. The data analysis for the varietal difference for germination and OD measured were analysed through the CRD design (Gopinath *et al.* 2020). The germination percentage values were converted to their arcsine before the analysis.

Fig. 1 SpeedySeed viability kit™.

RESULTS AND DISCUSSION

Viability assessment in paddy and lentil seed lots by using SpeedySeed viability kit: The comparison of the viability assessment by SpeedySeed viability kit and standard germination method was done in five varieties of paddy, namely PB 1121, PB 1509, PB 1692. PB 1886 and PB 1718. The total germination estimated by the standard germination test varied significant among the varieties, which varied from 98% to 46% (Table 1). The viability estimation was also done by using the SpeedySeed viability kit and compared with the viability estimation by the standard germination test (Table 1). For variety PB 1121, having the actual germination percentage of 98%, whereas the kit predicted the viability of 92% with the prediction accuracy of 93.87%. Variety PB 1509, recorded the actual viability of 95% and the viability estimated by the kit method was 87% with the prediction accuracy of 91.57%. The varieties

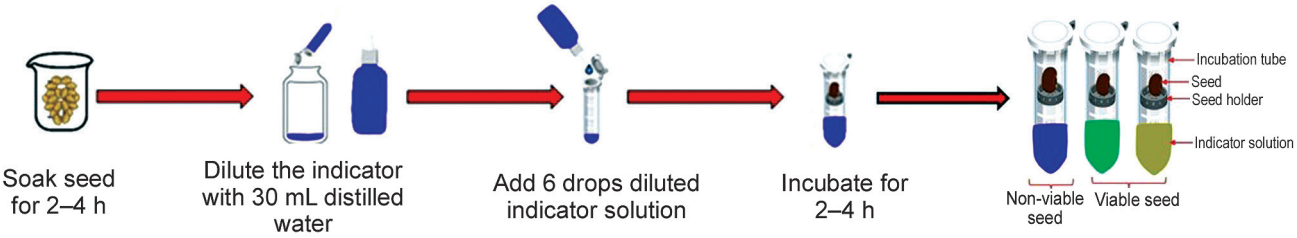

Fig. 2 Procedure of viability assessment using SpeedySeed viability kit, which consists of bromothymol blue reagent solution, 100 vials and seed holder.

Table 1 Comparison of viability assessment through standard germination test and SpeedySeed viability kit, and the optical density (OD) value in paddy varieties

Paddy varieties	Total germination %	Normal seedlings %	Viability estimated by kit %	Prediction accuracy % based on total G %	Deviation from Total G% (+/-)	Deviation from normal seedling % (+/-)	OD at 450 nm
PB 1121	98^{a} (81.87)*	90^{a} (71.57)	92^{a} (73.57)	93.87	6	2	1.69^{a}
PB 1509	95^{a} (76.64)	87^{ab} (68.58)	87^{b} (68.57)	91.57	8	0	1.62^{a}
PB 1692	88^{b} (69.43)	78^{b} (61.79)	77^{c} (61.58)	89.53	9	1	1.38^{b}
PB 1886	75^{c} (59.77)	66^{c} (52.73)	65^{d} (53.73)	89.04	8	1	1.26^{c}
PB 1718	46^{d} (42.70)	35^{d} (36.07)	41^{e} (39.96)	93.18	5	6	1.05^{d}
CD value	4.481	9.808	3.816				0.095
Method comparison	0.282^{NS}	0.18 ^{NS}					

*The values provided in parentheses correspond to arcsine-transformed data. Total germination % represents the proportion of all seeds that germinated. Normal seedling percentages were calculated in accordance with ISTA (2025) seedling evaluation standards. Distinct alphabetical letters indicate statistically significant variation among the varieties at the 5% significance level ($p< 0.05$). Method comparison was conducted between the viability estimated by kit method and total germination % and normal seedlings % by the standard germination test. CD, Critical difference; NS, Non-significant difference; OD, Optical density.

PB 1692 and PB 1886 having the actual viability 88% and 75%, respectively, whereas viability estimates by kit method were 77% and 65%, respectively, with prediction accuracy of 89.53% and 89.04%, respectively. The variety PB 1718 having the lowest actual germination percentage of 46% and the estimated viability by kit method was 41% with a prediction accuracy of 93.18%. Method comparison (Table 1) revealed a non-significant difference between the viability estimation methods (total germination by standard test vs. kit method, p=0.282), and a non-significant difference between the methods (normal seedlings vs. kit method, p=0.18). The viability estimated by kit method showed variation of 1–10% with the total viability by standard germination test (Table 1).

The viability assessment of five varieties of lentil, namely L-7903, IPL-220, PL-4, LL-699 and K-75 was done by both standard germination test and SpeedySeed viability kit. The total germination estimated by standard germination test varied significant among the varieties (Table 2). The germination of L-7903 and IPL-220 were statistically similar having the actual germination percentage of 99% and 97%, respectively. For these varieties, the viability estimated by kit method was 100% and 99%, respectively, with the prediction accuracy 99% and 98%, respectively. Variety PL-4 had actual viability of 93% and by the kit method 92% with the prediction accuracy of 99%. The variety LL-699 had actual viability of 89%, and an estimated viability by kit method was 90% with prediction accuracy of 99%. The

Table 2 Comparison of viability assessment through standard germination test and SpeedySeed viability kit, and the optical density (OD) value in lentil varieties

Lentil varieties	Total G %	Normal seedlings %	Viability estimated by Kit %	Prediction accuracy % based on total G %	Deviation from total G % (+/-)	Deviation from normal (+/-)	OD at 450 nm
L-7903	99^{a} (83.35)*	91^{a} (72.20)	100^{a} (90)	99	1	9	2.57^{a}
IPL-220	97^{a} (79.47)	77^{b} (61.11)	99^{a} (85.30)	98	2	22	2.44^{b}
PL-4	93^{ab} (74.28)	79^{bc} (62.49)	92^{b} (73.21)	99	1	20	2.18^{c}
LL-699	89^{b} (70.32)	65^{cd} (53.93)	90^{b} (71.56)	99	1	25	1.70^{d}
K-75	76^{c} (60.44)	55^{d} (48.06)	79^{c} (62.49)	96	3	24	1.67^{d}
CD value	7.96	11.58	4.06				0.093
Method comparison	0.44^{NS}	<0.001*					

*The values provided in parentheses correspond to arcsine-transformed data. Total germination (G%) represents the proportion of all seeds that germinated. Normal seedling percentages were calculated in accordance with ISTA (2025) seedling evaluation standards. Distinct alphabetical letters indicate statistically significant variation among the varieties at the 5% significance level ($p< 0.05$). Method comparison was conducted between the viability estimated by kit method and total germination % and normal seedlings % by the standard germination test. CD, Critical difference; NS, Non-significant difference; OD, Optical density.

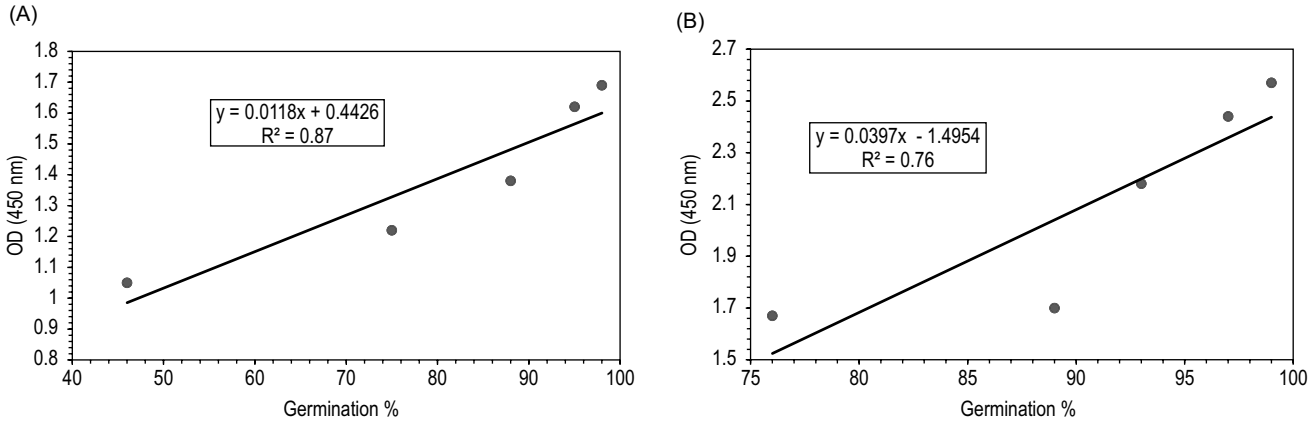

Fig. 3 Linear regression between the total germination by standard germination test and their corresponding optical density (OD) values of bromothymol blue solution, measured at 450 nm by using the spectrophotometer after the completion of incubation period in (A) Paddy seed lot and (B) Lentil seed lots.
Every observation reflects the mean calculated from three replicates (n = 3).

variety K-75 having the lowest actual germination of 76% and the estimated viability by kit method was 79% with the prediction accuracy of 96%. Method comparison (Table 2) revealed non-significant difference between the viability estimation methods (total germination by standard test vs. kit method; p=0.44), and a significant difference between the methods (normal seedlings vs. kit method; p<0.001). The viability estimated by kit method showed variation of 1–3% with the total viability by standard germination test (Table 2).

In this study, viability estimation in paddy and lentil seed lots was done by using SpeedySeed viability kit, with five seed lots in both crops. The estimated viability by kit method in this study closely matches with the total viability by the standard test (Table 1 and 2). The principle of kit is that viable and vigorous seed releases more CO_2 than the dead seeds or low-vigorous seeds. Respiration serves as a reliable indicator for assessing the viability and vigour (Bradford *et al.* 2013, Dode *et al.* 2013, De Sousa *et al.* 2018, Widjati *et al.* 2023). Dode *et al.* (2013) and De Sousa *et al.* (2018) estimated the vigour of soybean and okra seed lots, respectively by measuring seed respiration. In these studies, the respiration was related to the vigour of seed lot. CO_2 measurement by using the CO_2 sensor and IoT system was used to differentiate the seed lots of soybean with varying level of viability. Similarly, Min and Kang (2011) has developed a non-destructive, rapid colourimetric seed viability assay for Brassicaceae by using the resazurin reagent (RR), the colour change of RR will occur after 4 h incubation at 35°C, where the blue colour solution indicates the normal/viable seed and colourless solution indicates dead seed. SpeedySeed viability kit also estimates the viability calorimetrically, where blue colour of BTB solution indicates non-viable seed and yellow/green colour indicates the viable seeds at the end of the incubation period. The estimated viability results by kit method compared with the standard germination test, proved with prediction accuracy ranging from 89–93% in paddy and 96–99% in lentil.

Optical density measurement following the completion of incubation in paddy and lentil seed lots: The viability estimated using the kit method was validated by measuring the optical density (OD) of the BTB solution at 450 nm. In paddy, the OD values for varieties PB 1121 and PB 1509 were statistically similar, recorded at 1.69 unit and 1.62, respectively. Varieties PB 1692 and PB 1886 recorded OD values of 1.32 and 1.22, respectively. The variety PB 1718 having the lowest germination, showed an OD value of 1.05 (Table 1). These OD values differed significantly among the varieties (p<0.05). A linear regression analysis (Fig. 3A) between OD values and their corresponding germination percentages in paddy demonstrated a strong correlation, with an R^2 value of 0.87.

The OD values for lentil variety L-7903 was 2.57. For variety IPL-220, PL-4, and LL-69, the OD values were 2.44, 2.18, and 1.70, respectively. Variety, K-75 having the lowest germination, showed an OD value of 1.67 (Table 2). These OD values differed significantly among the varieties (p< 0.05). A linear regression analysis (Fig. 3B) between OD values and their corresponding germination percentages in lentil demonstrated a strong correlation, with an R^2 value of 0.76.

Seed lots with higher germination showed higher values of optical density compared to those with lower germination. Seed respiration measurement through CO_2 or O_2 sensors, are studied previously for evaluating the seed viability and vigour (Silva *et al.* 2021, Widajati *et al.* 2023). Currently, seed respiration of individual seed measurement by seed respiration analyser (Q2 technology) developed by ASTEC Global, was used to predict the seed viability and seed vigour (Powell 2017). The ASTEC values obtained by Q2 instruments describe several aspects of oxygen consumption including oxygen metabolism (percentage O2 consumed/ hour; OMR), the time required to reduce the initial oxygen level to 50% (R50), the area under the curve from time zero to 50% (AUC50) and the relative germination time. Based on the ASTEC values, it is claimed that the Q2 can reveal the 'complete picture of seed vigour' (ASTEC Global 2016). Study by Bradford *et al.* (2013) using seed lots of cabbage

(15 lots), the coefficients of determination (R^2) calculated of vigour, as expressed by the early germination count, and ASTEC values did not exceed 0.296. But Q2 instrument is costly, require skill to operate and cannot be used as on-farm viability testing. In a study conducted by Dalzeli and Tomilson (2017), where 12 species of angiosperm were tested, showed a significant positive relationship between seed viability and CO_2 evolution. In their study, linear regression plotted with viability and CO_2 evolution showed good correlation among the species tested, where good correlation with R^2 of 0.65 in onion, 0.59 in chilli, 0.88 in bean, and 0.92 in maize. The findings of our study also showed that CO_2 can be a reliable indicator for seed viability testing. As seeds respire, it releases CO_2 which is captured in a solution containing BTB, the dissolved CO_2 forms carbonic acid, thus lowering the *p*H of the solution. BTB is blue above 7.6 *p*H and green at neutral *p*H and yellow at *p*H < 6. The absorbance peak also varies with *p*H of the solution, with two peaks at 615 and 430 nm (Yang *et al.* 2023). Similarly, Min and Kang (2011) introduced a rapid, leachate-based method to assess the viability of individual radish seeds using resazurin dye. This approach leverages the deterioration of the seed membrane in aged seed lots, caused by reactive oxygen species (ROS) damaging cellular components such as mitochondria. The method effectively predicts seed germination through colourimetric analysis, with the highest OD at 570 nm observed in the most vigorous seeds.

Seed viability and vigour are critical determinants of crop establishment, yet conventional seed quality tests are time-consuming and require trained manpower and laboratory facilities. Standard germination tests may take two to three weeks, while the tetrazolium test, though faster, is technically demanding. The present study evaluated the SpeedySeed viability kit™ for seed viability assessment. Viability assessment of paddy and lentil seeds using the kit was completed within 6 h and 3 h, respectively. The viability estimates closely matched standard germination results, with prediction accuracies of 89–93% and 96–99% in paddy and lentil, respectively. Optical density measurements at 450 nm further validated the results, showing strong correlations with germination (R^2 = 0.87 in paddy and 0.76 in lentil). The SpeedySeed viability kit offers a rapid seed viability assessment by providing a simple, reliable, and cost-effective solution for farmers, seed companies, and researchers.

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