ASIAN JOURNAL OF AGRICULTURE
Volume 10, Number 1, June 2026
Pages: 10

E-ISSN: 2580-4537
https://doi.org/10.13057/asianjagric/g100124

Radicle emergence as a rapid indicator of sorghum seed vigor to predict mean germination time, vigor index, and field emergence

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Manuscript received: 17 November 2025. Revision accepted: 19 February 2026.

Abstract. *Kusumawardana A, Ilyas S, Qadir A, Trikoesoemaningtyas, Human S, Sihono. 2026. Radicle emergence as a rapid indicator of sorghum seed vigor to predict mean germination time, vigor index, and field emergence. Asian J Agric 10 (1): g100124. https://doi.org/10.13057/asianjagric/g100124.* This study aimed to develop a standardized radicle emergence test for sorghum (*Sorghum bicolor*) seeds by determining the optimal single-count timing and temperature for the simultaneous assessment of seed vigor and viability. This test was conducted by calculating the percentage of seeds whose radicles had emerged by at least 2 mm in length during a single observation period at the beginning of germination. Seed lots with low radicle emergence rates showed slow germination, reflecting an early physiological indicator of seed deterioration and a key factor contributing to reduced vigor. The experiment was arranged in a randomized complete block design with one factor: 12 sorghum genotypes, and four replications (blocks). Radicle length was measured at 2-h intervals from 40 to 48 h, with a minimum length of 2 mm. Results of radicle emergence (production of 2 mm radicle) at 25°C and alternating temperatures 20⇔30°C for 44 h (the optimal time), mean germination time, germination percentage, germination speed, vigor index, maximum growth potential, tetrazolium test, and field emergence were compared using 12 genotypes of sorghum seeds with standard germination above 83%. The single counts of radicle emergence performed at 25°C and 20⇔30°C were highly correlated with key vigor and viability indicators. Correlations with vigor-related traits, such as mean germination time (R^2=0.87-0.81), vigor index (R^2=0.83-0.89), germination speed (R^2=0.73-0.71), and field emergence (R^2=0.77-0.70), were strong, as were those with viability parameters, including standard germination (R^2=0.66-0.59) and tetrazolium test results (R^2=0.58-0.52). This study concludes that a single count of radicle emergence at 44 h, conducted at either 25°C or 20⇔30°C, is a method for simultaneous assessment of seed vigor (mean germination time, vigor index, germination speed, and field emergence) and viability in sorghum, offering a significant improvement over more time-consuming tests, such as germination test that requires 10 days.

Keywords: Germination percentage, germination speed, seed deterioration, tetrazolium test, viability

INTRODUCTION

Sorghum is a suitable food crop for cultivation in Indonesia. Sorghum can serve as an alternative carbohydrate source (Batog et al. 2020) and is used as animal feed and as a bioethanol feedstock (Human et al. 2023). Sorghum is classified as a C4 plant and is efficient at producing high levels of photosynthetic products (Amaducci et al. 2016), tolerant to drought and waterlogging conditions, and relatively resistant to pests and diseases (Habyarimana et al. 2020).

Seed quality is most important for producing vigorous and healthy plants in the field. A key component of crop seed performance in the field largely depends on seed vigor (Finch-Savage and Bassel 2016; Wen et al. 2018). High-quality seeds are characterized by high levels of purity, viability, vigor, and are free from seed-borne pathogens. Vigor testing is a more sensitive seed quality index for detecting seed deterioration than germination testing (Ilyas 2012). Conventional seed certification and testing processes are time-consuming, which is problematic given sorghum seeds' notoriously short shelf life and rapid deterioration within four months (Mbughi et al. 2024). According to the Ministry of Agriculture (2023), the expiration period for certified sorghum seeds is four months after testing is completed. The short shelf life of sorghum seeds is often an obstacle to seed certification.

Radicle emergence is a recent vigor test correlated with seedling emergence potential at various environmental conditions in a wide range of sowing conditions (Matthews and Powell 2011; Demir et al. 2020a; Ozden et al. 2020). ISTA has validated the radicle emergence test to assess seed vigor in maize, radish, oilseed rape, and wheat (ISTA 2021). This test is conducted by calculating the percentage of seeds whose radicles have emerged by at least 2 mm in length during a single observation period at the beginning of germination. Seed lots with low radicle emergence rates showed slow germination, reflecting an early physiological indicator of seed deterioration and a key factor contributing to reduced vigor. The delayed radicle emergence is primarily attributed to the seed aging processes (Eren et al. 2023). Yin et al. (2018) state that the speed of radicle emergence is closely related to the level of starch mobilization in seeds. The radicle emergence test was originally developed for corn seeds by Matthews and Khajeh-Hosseini (2006), establishing observation periods of 66 hours post-germination at 20°C and 144 hours at 13°C; these have been recommended by ISTA since 2014. The optimum radicle emergence count time varies among the species in relation to the prediction of seedling emergence: for example, 120 h at 20°C in leek (Ermis et al. 2015), 52 h at 20°C in *Avena sativa* and 76 h at 25°C in *Elymus nutans* (Lv et al. 2016), 42 h at 25°C in soybean (Astuti et al. 2020), 48 h at 20°C in petunia (Demir et al. 2020a), 49 h at 20⇔30°C in marigold (Ilbi et al. 2020), 24 h at 20°C in rocket (Ozden et al. 2020), 20 h at 20°C in Chinese milk vetch (Tao et al. 2020), 72 h at 20°C and 68 h at 25°C in shallot (Kamanga et al. 2021), 51 h at 25°C in corn (Khusna et al. 2021), 54 h at 20°C in long bean (Budiman et al. 2024), and 36 h at 28°C in sorghum (Diaguna et al. 2024).

The radicle emergence test is characterised by its brevity compared to other ISTA-validated methods, such as accelerated aging and controlled deterioration, as the radicle emergence count is typically measured within 24-72 h of germination onset (Powell 2022). Radicle emergence serves as a dual-purpose measure, capable of conducting the test during germination and providing results for both tests (Powell 2022). In previous studies, the radicle emergence test for sorghum seeds was established at 28°C after 36 h (Diaguna et al. 2024). Our study advances that work by developing and validating a radicle emergence test specifically at the ISTA-prescribed germination temperatures of 25°C constant and 20⇔30°C alternating. This alignment with standardized testing conditions enables the radicle emergence test to function as a dual-purpose assessment, providing rapid vigor predictions while simultaneously integrating with official germination testing procedures.

Based on the identified need for a rapid and reliable vigor assessment method while following the ISTA's temperatures for germination tests, this study aimed to develop a rapid vigor test method for sorghum seeds by determining the appropriate time for radicle emergence at a constant temperature of 25°C and alternating temperatures of 20⇔30°C. We hypothesized that the radicle emergence test would correlate with other vigor measurement tests.

MATERIALS AND METHODS

Research sites

The experiment was conducted at the Seed Quality Testing Development Center for Food Crops and Horticulture (BBPPMBTPH) of the Ministry of Agriculture, Indonesia, and Leuwikopo Experimental Farm, Institut Pertanian Bogor, Ciampea Sub-district, Bogor, West Java, Indonesia, at 6°33'S and 106°43'E, 190 m asl. This study was conducted from January to April 2025.

Sorghum seeds

Sorghum seeds used in this study were obtained from the Food Crop Research Center (PRTP), the Organization of Agricultural and Food Research (ORPP) of the National Research and Innovation Agency (BRIN) of the Republic Indonesia. Twelve genotypes were used: nine BRIN sorghum lines (GHP-2, GHP-16, CTY-43, GH-1, GH-7, GH-9, G-5, G-7, and G-8) and three national varieties (Bioguma 1, Pahat, and Samurai 2). Sorghum seeds of 12 genotypes were harvested in November 2025 from the Leuwikopo Experimental Farm, Institut Pertanian Bogor. All sorghum genotypes tested had high standard laboratory germination (>83%, normal germination). Seeds were placed in paper bags and stored at 25±2°C and ≤60% humidity until used for laboratory testing (approximately two months). This short-term ambient storage reflects the typical post-harvest handling and distribution period in the sorghum seed supply chain, during which the initial decline in vigor often begins.

Procedures

Experimental design

The experiment was arranged in a randomized complete block design with one factor. The factor level given was 12 sorghum genotypes with four replicates (each comprising 100 seeds), resulting in 48 experimental units.

Seed quality testing

The radicle emergence test was performed using the top-of-paper method. Seeds were positioned within a 9 cm diameter petri dish covered with a moistened paper towel using 10 mL of distilled water. Seeds were incubated in a germinator with constant temperatures of 25°C and alternating temperatures of 20⇔30°C (the symbols ⇔ indicate alternating temperature regimes: 20°C for 16 h and 30°C for 8 h). Radicle emergence was calculated as follows: (number of radicles with a minimum length of 2 mm)/number of seeds) × 100%. Radicle length was measured at 2 h intervals spanning the time frame from 40 to 48 h (Diaguna et al. 2024). The seed moisture content was measured using a high-temperature oven method at 130-133°C for 4 h (ISTA 2021). Each sample was repeated four times, with a weight of 4.5±0.5 g. The germination percentage was calculated by adding the percentage of normal seedlings of the first count at 4 days after planting (DAP) and the final count observed 10 DAP. Germination speed was measured by summing the percentage increase in normal seedlings per etmal (24 h) from the first day to 10 days after sowing. The vigor index was defined as the percentage of normal seedlings at the first count (4 days). The Maximum Growth Potential (MGP) was the total number of seeds that germinated (normal and abnormal seedlings) on the last day of observation, divided by the total number of seeds planted, and multiplied by 100%. MGP was calculated as follows: (number of normal seedlings at 4 DAP + number of normal seedlings at 10 DAP + number of abnormal seedlings at 10 DAP)/number of seeds) × 100%. The tetrazolium test was performed as follows: the seeds were moistened for 18 h at 7°C, and then longitudinally cut through the embryo and 1/4 of the endosperm. They were then immersed in 1% tetrazolium solution at 30±2°C for 3 h. The maximum area of the unstained embryo considered viable was 1/3 of the radicle measured from the radicle tip (ISTA 2021). Mean emergence time was calculated as the average time from seed imbibition to radicle emergence. Field emergence was calculated as the percentage of normal seedlings in the field 4 weeks after planting. The seeds were planted in plots measuring 1×2.8 m with a planting distance of 70×20 cm. The average daily temperature during the study was 25-27°C.

Data analysis

Data were analyzed using Analysis of Variance (ANOVA) at the 5% level in SAS software. Before analysis, the data were examined to confirm that the assumptions of normality and homogeneity of variances required for ANOVA were satisfied. If the test results showed a significant effect, further tests were done using Duncan's Multiple Range Test (DMRT) at α=5%. Moreover, correlation coefficient (r) values were determined between radicle emergence and germination percentage, mean germination time, vigor index, germination speed, tetrazolium test, maximum growth potential, and field emergence. Finally, regression coefficients (R^2) were also calculated.

RESULTS AND DISCUSSION

Laboratory analysis of seed quality showed significant differences among 12 sorghum genotypes in terms of germination percentage, germination speed, vigor index, maximum growth potential, and tetrazolium test results, but no significant differences in moisture content (Table 1). According to the regulatory standard (Ministry of Agriculture 2023), the minimum germination percentage of sorghum seeds for distribution is 70%, with a maximum moisture content of 12%. In this study, the germination percentage value ranged from 83.75 to 96.75% with an average moisture content of 12%.

As shown in Table 1, Bioguma-1, Pahat, GH-9, G-5, and G-7 consistently demonstrated superior seed quality. These genotypes not only achieved the highest final germination percentages (94.5-96.75%) but also exhibited the fastest germination speed. The combination of high and rapid germination is a key indicator of strong seed lot performance. Their excellence was further confirmed by the tetrazolium test, which showed near-perfect seed viability (97.25-98.75%), indicating that the high germination results were due to a high proportion of physiologically sound seeds. Although germination tests are conducted under ideal conditions, the vigor index is a more sensitive measure that predicts performance under stressful field conditions; here, a clear hierarchy emerged. Genotypes G-5, G-7, and GH-9 also registered the highest vigor values (84.00-87.50%).

The fastest observation time should be selected to save testing time. Radicle emergence was first recorded at 30 h after incubation, and radicle length was between 1 and 2 mm at 38 h. The radicle length of more than 2 mm was identified between 40 h and 48 h. An increase in radicle length of more than 2 mm began at 42 h and peaked at 46 h. The cumulative radicle emergence determined at 25°C and 20⇔30°C for 12 seed lots of sorghum is shown in Figure 1.

In Table 2, the radicle emergence observation time was determined based on the highest correlation value (r) and coefficient of determination (R^2) between percentages of germination (normal seedlings) and radicle emergence at temperatures of 25°C and 20⇔30°C. A linear correlation was observed between radicle emergence and germination percentage at 42, 44, and 46 h (Table 2). A significant and fairly strong correlation between radicle emergence and germination percentage occurred during the 44-hour germination period at 25°C with an R^2 value of 0.66 and r=0.81, with the equation $y = 0.39x + 66.53$ and the 44-hour germination period at 20⇔30°C with an R^2 value of 0.59 and r = 0.76, with the equation $y = 0.43x + 65.20$.

Table 1. Seed quality of twelve sorghum genotypes

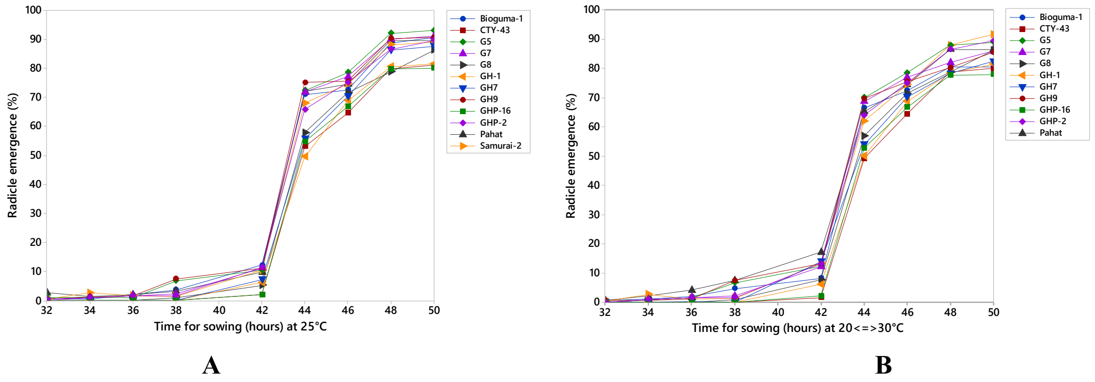
Genotype	Moisture content (%)	Germination percentage (%)	Germination speed (% /d)	Vigor index (%)	Maximum growth potential (%)	Tetrazolium test (%)
Bioguma-1	12.1	96.75a	26.83a	80.50c	97.00a	98.25a
Pahat	12.1	96.50a	27.08a	81.00c	96.50ab	98.25a
Samurai-2	12.0	91.75b	24.25b	78.25cd	91.75c	95.50b
GHP-2	12.0	89.50c	23.51bc	76.00d	89.50cd	94.50bc
GHP-16	12.1	87.50c	20.31d	65.00g	89.00d	90.50d
CTY-43	12.0	88.50c	21.57cd	64.75g	88.50d	93.50c
GH-1	12.0	88.25c	22.23cd	64.25g	88.25d	93.75bc
GH-7	12.1	89.50c	22.17cd	68.25g	90.00cd	94.50bc
GH-9	12.0	96.00a	27.68a	84.00f	96.00ab	98.50a
G-5	12.0	95.75a	26.94a	86.75ab	95.75ab	98.75a
G-7	12.0	94.50a	26.80a	87.50a	94.50b	97.25a
G-8	12.1	83.75d	22.12cd	72.25e	84.25e	90.25d
Average	12.0	91.52	24.29	75.70	91.75	95.29

Note: In the column, any means with a common letter are not significantly different from each other ($p<0.05$)

Table 2. Linear correlation of radicle emergence at different germination periods

Germination period (hours)	Linear correlation					
	constant temperatures of 25°C			alternating temperatures of 20⇔30°C		
	R^2	r	Linear equation	R^2	r	Linear equation
42	0.62	0.78	y = 0.45x + 85.62	0.34	0.58	y = 0.33x + 87.29
44	0.66	0.81	y = 0.39x + 66.53	0.59	0.76	y = 0.43x + 65.20
46	0.11	0.33	y = -0.32x + 117.65	0.00	0.01	y = -0.01x + 93.04

Note: x: Radicle emergence, y: Germination percentage, $R^{2:}$ Coefficient of determination, r: Correlation coefficient



A **B**

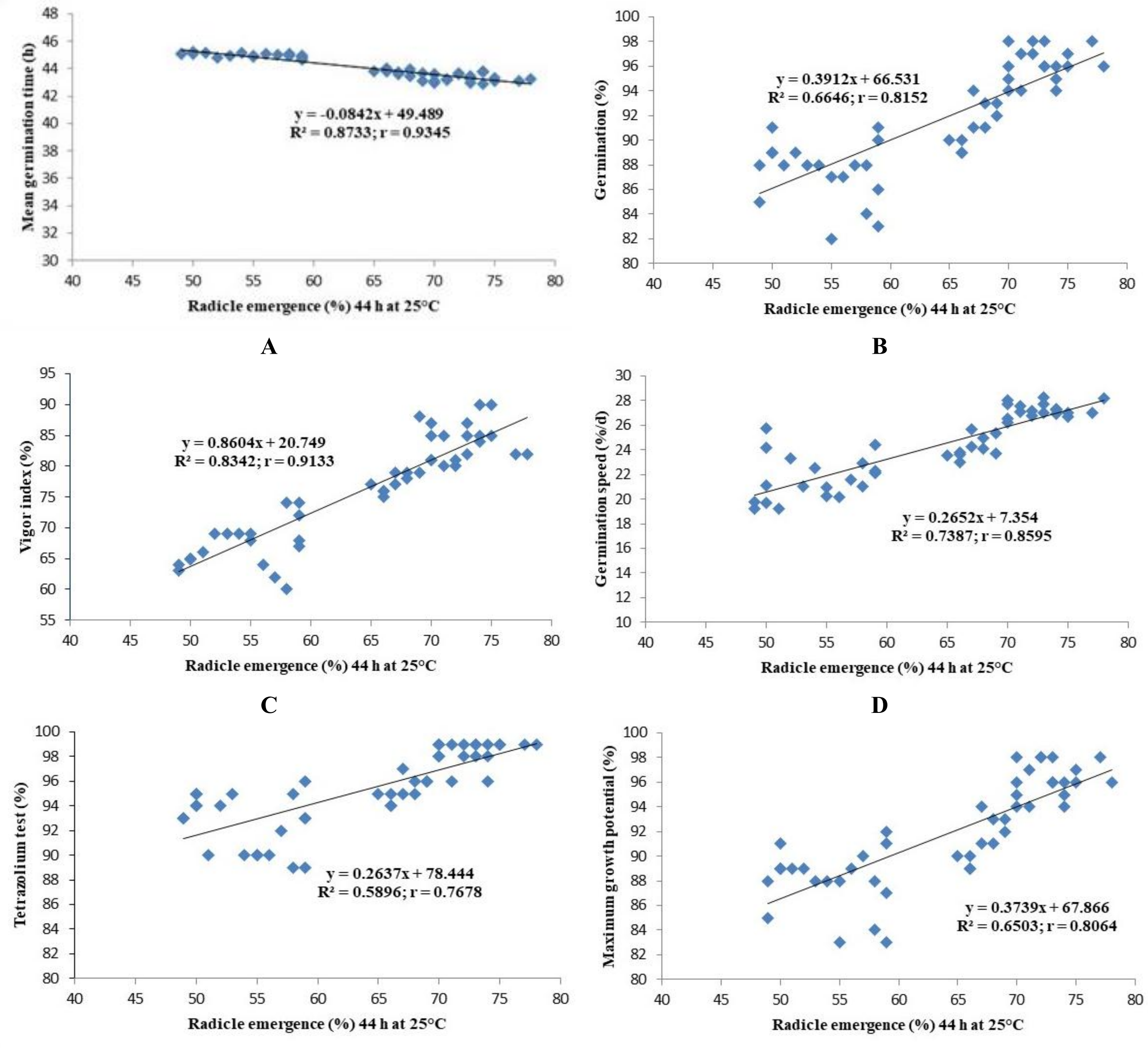
Figure 1. Progress of radicle emergence of twelve sorghum seed lots at: A. 25°C, B. 20⇔30°C

A correlation coefficient (r) value close to 1 or -1 indicates a stronger linear relationship between the two variables. A value of $0<r<0.5$ indicates a weak correlation, $r=0.5$ indicates a moderate correlation, and $0.5<r<1$ indicates a strong correlation. Therefore, the recommended observation time for radicle emergence in sorghum seeds is 44 h at both 25°C and 20⇔30°C. Based on this, regression and correlation analyses were performed with several viability and vigor measures at the 44-hour observation time. The results of the regression analysis on various viability and vigor measures at the 44-hour observation time showed a relatively high correlation coefficient at 25°C ($r=0.81$) and 20⇔30°C ($r=0.76$). The coefficient of determination is a measure describing the model's ability to explain the Y values; in this case, various physiological quality measures of seeds. The higher the R^2 value, the better the model explains the behavior of variable Y. The linear relationships between the 44-hour radicle emergence and various vigor tests are presented in Figure 2 for a temperature of 25°C and Figure 3 for a temperature of 20⇔30°C.

Radicle emergence at 44 h showed a strong negative relationship with mean germination time under both temperature regimes (Figures 2.A and 3.A). At 25°C, radicle emergence explained 87% of the variation in mean germination time ($R^2=0.87$, $p<0.001$) with the regression equation $y = -0.0842x + 49.4894$, whereas at alternating temperatures of 20⇔30°C, 81% of the variation was explained ($R^2=0.81$, $p<0.001$) with the equation $y = -0.0877x + 49.5858$. In both cases, increased radicle emergence was associated with a shorter mean germination time, with each 1% increase in radicle emergence reducing mean germination time by approximately 0.08 h. These results indicate that early radicle protrusion is a strong predictor of faster and more synchronized germination

Radicle emergence at 44 h showed a consistent positive linear relationship with germination percentage, germination speed, vigor index, maximum growth potential, and tetrazolium test values. Regarding the germination percentage, radicle emergence accounted for 66% of the variation at 25°C ($y = 0.39x + 66.53$; $R^2=0.66$, $p<0.001$) and 59% at alternating temperatures of 20⇔30°C ($y = 0.43x + 65.20$; $R^2=0.59$, $p<0.001$), indicating that higher radicle emergence reliably corresponded to higher final germination.

The vigor index, which assesses the overall robustness and growth potential of the seedling, was profoundly influenced by the initial radicle emergence. Rapid and robust radicle emergence provides the basis for vigorous seedling growth. Germination speed, a measure of the synchronicity and rapidity of germination, is intrinsically linked to the timing of radicle emergence. The data strongly support this; genotypes with higher and more consistent radicle emergence values consistently exhibited faster germination. Radicle emergence showed particularly strong relationships with vigor index and germination speed. Regression analysis indicated that radicle emergence explained 83% (25°C) and 89% (20⇔30°C) of the variation in vigor index, and 73% and 71% of the variation in germination speed at the respective temperatures ($p<0.001$). Radicle emergence at 44 h under 25°C accounted for 58% of the variation in tetrazolium test values and 65% of the variation in maximum growth potential. The 44 h radicle emergence value at temperatures of 20⇔30°C explains 52% of the variance in tetrazolium test values, and 57% of the variance in maximum growth potential values.



A B C D E F

Figure 2. Linear correlation between radicle emergence at 25°C after 44 hours. A. Mean germination time, B. Germination percentage, C. Vigor index, D. Germination speed, E. Tetrazolium test, and F. Maximum growth potential

Figure 4 shows a positive linear relationship between radicle emergence and field emergence. This means that the higher the radicle emergence value, the higher the field emergence value. Radicle emergence values could explain 77% of the variation in field emergence values with a linear equation estimator $y = 0.4148x + 66.020$ at 25°C (R^2=0.77, $p<0.001$) and 70% of the variation with a linear equation estimator $y = 0.4622x + 64.426$ at 20⇔30°C (R^2=0.70, $p<0.001$).

Discussion

The study found a close relationship among radicle emergence, germination percentage, germination speed, vigor index, maximum growth potential, tetrazolium test, and field emergence. The radicle emergence test (a minimum length of 2 mm) is an effective method for assessing sorghum seed viability. Under the tested conditions (44 h at 25°C and 44 h at 20⇔30°C), seed lots that germinated faster (lower mean germination time) had a higher emergence rate. This strong correlation supports the findings of Cheshmi and Khajeh-Hosseini (2020). The negative correlation indicates that a lower radicle emergence was associated with a higher mean germination time as the seed deteriorated (Nugraheni et al. 2023). Mean germination time represents the average duration required for seeds to progress from imbibition to radicle emergence. In corn, this parameter showed a close relationship with radicle emergence observed after 66 hours at 20°C and 6 days at 13°C (Matthews and Powell 2011). The 44 hours after germination was chosen because this period has a strong correlation and a high coefficient of determination with the mean germination time at both 25°C and

20⇔30°C (R^2=0.87 and 0.81, respectively). According to Khusna et al. (2021), the determination of optimal observation time for radicle emergence was guided by its strong correlation and coefficient of determination (R^2) with mean germination time. Hoque et al. (2022) also showed that the mean germination time was closely correlated with radicle emergence in rice seeds at the selected observation time, 72 h at 22°C. A higher proportion of abnormal seedlings and slower radicle emergence is a direct sign of seed aging in safflower (Tonguç et al. 2023) and onion (Demir et al. 2020b), while slower germination (high mean germination time) has been linked to seed aging in several other crop species (Matthews and Powell 2011).

In seed quality testing, germination percentage is commonly used to determine seed quality. However, analyzing normal seedlings during germination testing is subjective and not easy. Therefore, it is necessary to develop a more objective testing method, namely, using radicle emergence. The estimation equations $y = 0.39x + 66.53$ (constant temperatures 25°C) and $y = 0.43x + 65.20$ (alternating temperatures 20⇔30°C) reinforce this functional relationship; each unit increase in radicle emergence percentage consistently predicts an increase in germination percentage. Therefore, the radicle emergence test can predict germination. This study aimed to develop a rapid seed quality test to classify sorghum germination capacity, thereby allowing an analyst to monitor hundreds of germination trials.

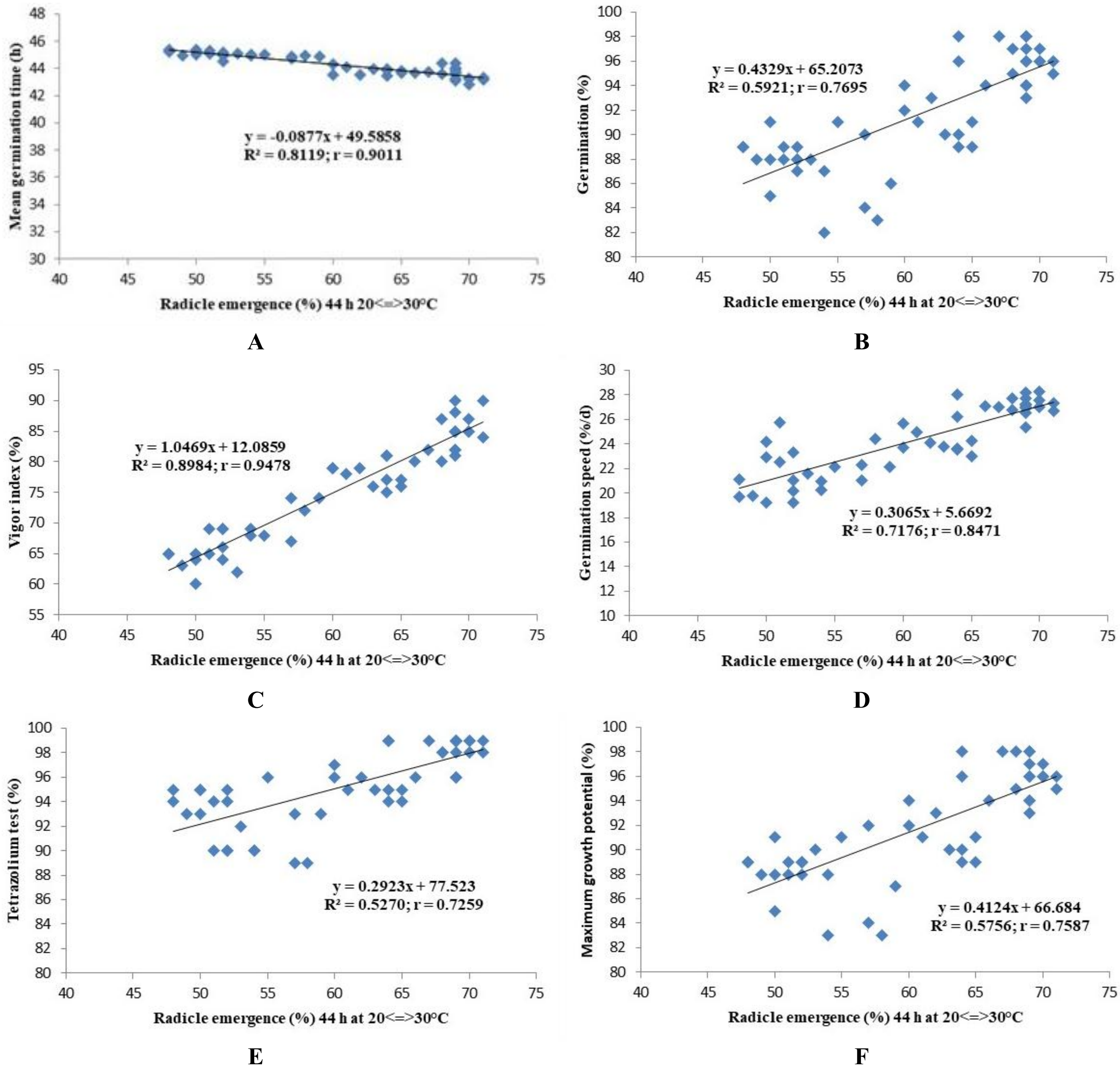


Figure 3. Linear correlation between radicle emergence at alternating temperatures 20⇔30°C after 44 hours. A. Mean germination time, B. Germination percentage, C. Vigor index, D. Germination speed, E. Tetrazolium test, and F. Maximum growth potential

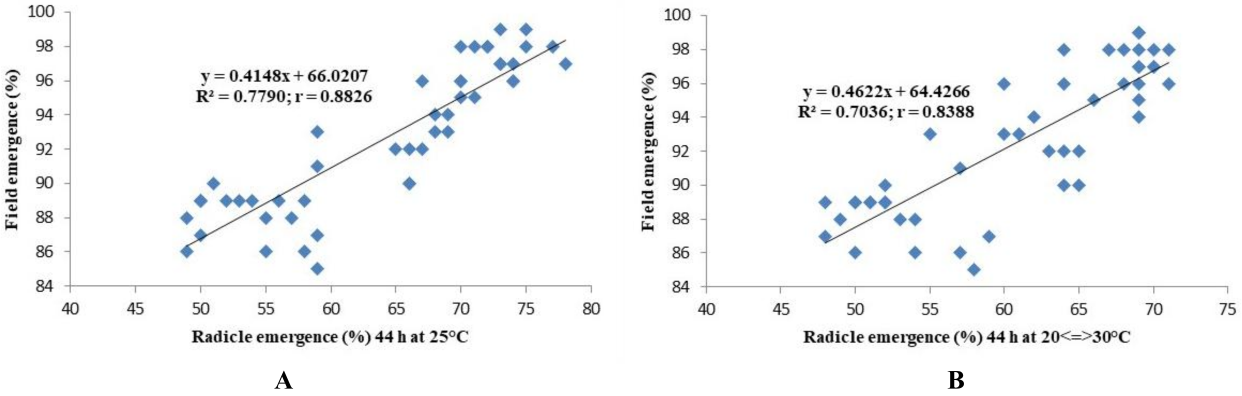


Figure 4. A. Linear correlation between radicle emergence at 25°C after 44 hours and field emergence, B. Linear correlation between radicle emergence at alternating temperatures 20⇔30°C after 44 hours and field emergence

According to Mavi et al. (2016), the germination test is time-intensive and may not accurately reflect seed lot performance under suboptimal field environments. Ozden et al. (2018) identified the optimal observation time for radicle emergence by selecting the point with the highest coefficient of determination (R^2) in a linear regression between radicle emergence and the germination percentage of eggplant seeds. According to Demir et al. (2020b), the radicle emergence after 80 h of germination correlates significantly with the number of normal seedlings in onion seeds. Radicle emergence at 25 °C and 20⇔30°C only took 44 h (less than 2 days), which is faster than the germination percentage test, which took 10 days. Therefore, radicle emergence can be an alternative to the germination test for sorghum seeds. The radicle emergence test, as an early indicator of germination, can be a reliable, fast, and efficient alternative, making it very useful in commercial seed testing that requires quick results (Ilyas and Widajati 2025). Previous studies have demonstrated that the radicle emergence test can predict germination percentages across various seed species: 42 h at 25°C in soybean (Astuti et al. 2020), 72 h at 20°C and 68 h at 25°C in shallot (Kamanga et al. 2021), 51 h at 25°C in corn (Khusna et al. 2021), 54 h at 20°C in long bean (Budiman et al. 2024), and 36 h at 28°C in sorghum (Diaguna et al. 2024).

Analysis of 12 sorghum seed lots revealed a significant positive correlation between radicle length and vigor index. A high level of radicle emergence indicates that the seeds have better germination speed and uniformity, which is an important indicator of high seed vigor. Therefore, seeds with high radicle emergence performed better in the field than seeds with low radicle emergence, even if both have the same germination percentage. The derived regression equation allows the accurate estimation of the vigor index based on the radicle emergence at 44-hour measurement. This is in accordance with Diaguna et al. (2024), who reported that the radicle emergence test method for sorghum seed, which was established at a temperature of 28°C after a period of 36 h, has been demonstrated to predict the vigor index. In our study, the high R^2 value, implying a strong correlation between the 44-hour radicle emergence percentage and the vigor index, is not merely a statistical point; it is a finding with profound practical implications for seed technology. The vigor index is a composite result, often measured later in the germination process. A high R^2 with the 44-hour test suggests that the fate of a seed lot's vigor is largely determined within the first 44 h. This has transformed the radicle emergence test from a simple observation to a powerful, early diagnostic tool. Seed technologists can predict the final vigor index result days in advance, drastically speeding up quality control and decision-making.

Radicle emergence and tetrazolium tests are complementary seed quality tests that measure different aspects of seed physiology. The tetrazolium test measures seed viability (potential for life), whereas the radicle emergence test measures seed vigor (expression of life). While the tetrazolium test reveals aging through tissue staining, radicle emergence measures differences in germination rate (Van der Walt and Witkowski 2017; Kusumawardana et al. 2018; Ferro et al. 2019; Sales et al. 2022). Seed viability is affected by its own degree of aging, and tetrazolium tests can identify seed viability based on differences in germination rate due to aging (Wang et al. 2023). This study found a highly significant relationship between radicle emergence and tetrazolium test results in sorghum, confirming the effectiveness of tetrazolium tests in detecting vigor differences. This aligns with studies on other species, such as wheat, further supporting the use of radicle emergence for vigor testing. Significant relationships between radicle emergence and other vigor tests were also found in other species. Guan et al. (2018) indicated that radicle emergence after 48 h at 20°C was significantly related to differences in vigor, measured as germination energy, germination index, vigor index, complex stress vigor test, and field emergence in lots of wheat seeds.

Seed vigor reflects several characteristics that determine seed quality and the potential for crop uniformity across a wide range of environmental variables (Finch-Savage and Bassel 2016). Numerous studies have demonstrated that radicle emergence serves as a critical indicator of seed vigor. A delay in reaching 2 mm radicle length in low-vigor seeds compared to high-vigor seeds is interpreted as reduced metabolic activity. This impaired metabolism slows the initial response to water uptake (imbibition) and the subsequent activation of critical processes, such as DNA repair and reserve mobilization (Matthews and Powell 2011), a phenomenon also observed by Mavi et al. (2016). Thus, the radicle emergence test serves as a dependable method for assessing seed vigor, accurately predicting field emergence, and evaluating the speed and uniformity of early germination. According to Reed et al. (2022), vigorous seeds are characterized by rapid germination, completing the process within a relatively short period, while seeds of lower vigor require more time to germinate.

The observations in this study indicated that the radicle emergence count at 44 h at either 25°C or 20⇔30°C can be used to predict field seed emergence. These significant coefficients of determination between radicle emergence and field emergence show that a large proportion of the variance in seedling emergence can be explained by radicle emergence (Figure 3). Finch-Savage and Bassel (2016) stated that seed vigor is considered a potential seedling emergence/stand establishment in the field or transplant modules, particularly when sowing environments are suboptimal. Radicle emergence counts offer a faster assessment of a seed lot's capacity to generate vigorous seedlings under suboptimal conditions. The radicle emergence test will help distinguish a seed lot that has better seedling production potential under suboptimal conditions. The prediction of seedling emergence from a single radicle count is supported by previous findings showing that radicle emergence can identify differences in seedling emergence and growth potential in rocket seed lots (Ozden et al. 2020). Study on onion seeds, Kamanga et al. (2021), indicated that a single measurement of radicle emergence taken at 72 h (at 20±1) and 68 h (at 25±1) could reliably forecast both the laboratory germination rate observed 12 days after sowing and the field emergence recorded 14 days after planting. Similarly, the RE test in soybean at 25±2°C observed after 42 h ± 15 min was closely correlated and could predict percentage and speed of germination, vigor index, field emergence, and mean germination time (Astuti et al. 2020).

The radicle emergence test is not merely about observing germination; it is a precise assessment of the completion of Phase II and the critical transition to Phase III of seed germination (Bewley et al. 2013). Bai et al. (2021) stated that seed germination is a triphasic process of water uptake consisting of Phase I, an initial rapid imbibition; Phase II, a lag phase during which metabolism is activated; and Phase III, a second rapid water uptake that leads to radicle emergence. This sequence of water absorption is driven by metabolic and physiological changes within the seed, culminating in germination with the emergence of the radicle. The radicle emergence test was fundamentally a test for the successful completion of Phase II. A seed that has imbibed water (Phase I) but fails to initiate radicle emergence has stalled in Phase II due to a physiological deficiency.

A limitation of this study is that radicle emergence assessments were conducted under controlled laboratory conditions, which may not fully replicate the complex biotic and abiotic stresses, such as soil crusting, pathogen pressure, and temperature fluctuations that seeds encounter in a field environment. In addition, manual counting of radicle emergence is not easy when a large number of lots are considered. Future research should focus on the correlation between radicle emergence and field performance under stress conditions and integrate radicle emergence tests for seed sorghum with digital imaging analysis to further enhance the objectivity and scalability of large-scale seed lot evaluations. In our study, radicle emergence distinguished the seedling production potential of seed lots, with germination above 83% of all 12 genotypes. This indicates that radicle emergence distinguishes seed lots with high germination percentages in terms of seed vigor, aligning with the ISTA definition of seed vigor (ISTA 2021): the sum of properties that determine the activity and performance of seed lots with acceptable germination in a wide range of environments. Vigor tests are expected to complement standard laboratory germination tests in reflecting the potential to produce high emergence percentages and seed longevity (Matthews and Powell 2011). An ideal seed vigor test should be reproducible, rapid, and consistently applicable across laboratories. In this study, radicle emergence measured at 44 h was identified as the most effective vigor test. Results can be obtained within 2 days, which is faster than the vigor index (4 days) and the germination percentage (10 days).

This study concludes that the radicle emergence test, conducted over 44 h at either 25°C or 20⇔30°C, is a validated and recommended vigor test for sorghum. A single radicle emergence count conducted 44 h after imbibition under either 25°C or alternating 20⇔30°C conditions showed strong and consistent relationships with key laboratory and field performance parameters. Radicle emergence at 44 h explained 87% and 81% of the variation in mean germination time, 83% and 89% of the variation in vigor index, and 73% and 71% of the variation in germination speed at 25°C and 20⇔30°C, respectively. Moderate to strong predictive relationships were also observed with germination percentage (R^2=0.66 and 0.59), maximum growth potential (R^2=0.65 and 0.57), tetrazolium test results (R^2=0.58 and 0.52), and field emergence (R^2=0.77 and 0.70). These results confirm that early radicle emergence reflects both the physiological status of the seed and its capacity to establish seedlings under field conditions. Importantly, the radicle emergence test reduced the assessment time to less than 2 days, compared with 10 days required for standard germination testing, offering substantial efficiency gains for seed quality evaluation. Given its speed, reproducibility, and strong predictive relationships with established vigor and field emergence parameters, the 44-hour radicle emergence test has clear potential to be considered as a complementary method within ISTA guidelines and national sorghum seed testing protocols.

ACKNOWLEDGEMENTS

The authors would like to express their gratitude to the Directorate General of Research and Development, Ministry of Higher Education, Science, and Technology of the Republic of Indonesia, for funding support through the Postgraduate Research Scheme for Doctoral Dissertation Research (PDD), on behalf of Prof. Satriyas Ilyas, PhD, under contract No.027/E5/PG.02.00.PL/2024 Fiscal Year 2024.

REFERENCES

Amaducci S, Colauzzi M, Battini F, Fracasso, Perego A. 2016. Effect of irrigation and nitrogen fertilization on the production of biogas from maize and sorghum in a water-limited environment. Eur J Agron 76: 54-56. https://doi.org/10.1016/j.eja.2016.01.019.

Astuti F, Budiman C, Ilyas S. 2020. Pengembangan metode uji cepat vigor benih kedelai dengan pemunculan radikula. Indones J Agron 48 (2): 135-141. https://doi.org/10.24831/jai.v48i2.29635. [Indonesian]

Bai B, van der Horst N, Cordewener JH, America AHP, Nijveen H, Bentsink L. 2021. Delayed protein changes during seed germination. Front Plant Sci 12: 735719. https://doi.org/10.3389/fpls.2021.735719.

Batog J, Frankowski J, Wawro A, Lacka A. 2020. Bioethanol production from the biomass of selected sorghum varieties cultivated as the main and second crop. Energies 13 (23): 6291. https://doi.org/10.3390/en13236291.

Bewley JD, Bradford KJ, Hilhorst HWM, Nonogaki H. 2013. Seeds: Physiology of Development, Germination and Dormancy. Springer, New York. https://doi.org/10.1007/978-1-4614-4693-4.

Budiman C, Nurhasanah F, Ilyas S, Qadir A, Palupi ER, Zamzami A, Diaguna R. 2024. Pengembangan metode uji cepat vigor benih kacang panjang (*Vigna sinensis* l.) melalui pemunculan radikula menggunakan pengolahan citra digital. Jurnal Hortikultura Indonesia 15 (1): 42-48. https://doi.org/ 10.29244/jhi.15.1.42-48. [Indonesian]

Cheshmi M, Khajeh-Hosseini M. 2020. Single count of radicle emergence, DNA replication during seed germination, and vigour in alfalfa seed lots. Seed Sci Technol 48 (3): 367-380. https://doi.org/10.15258/sst.2020.48.3.05.

Demir I, Erturk N, Gokdas Z. 2020a. Seed vigour evaluation in petunia seed lots to predict seedling emergence and longevity. Seed Sci Technol 48 (3): 391-400. https://doi.org/10.15258/sst.2020.48.3.07.

Demir I, Ozden E, Gokdas Z, Njie SE, Aydın M. 2020b. Radicle emergence test predicts normal germination percentages of onion seed lots with different cultivars and genotypes. Mustafa Kemal Üniversitesi Tarım Bilimleri Dergisi 25 (3): 434-442. https://doi.org/10.37908/mkutbd.697450.

Diaguna R, Widajati E, Permatasari OSI, Suhartanto MR, Suwarno PM, Budiman C, Saroza AR, Fuad H. 2024. Radicle emergence test method for estimating sorghum seed quality: A tropics practice. J Stored Prod Res 105: 102263. https://doi.org/10.1016/j.jspr.2024.102263.

Eren E, Ermis S, Oktem G, Demir I. 2023. Seed longevity potential predicted by radicle emergence vigor test in watermelon seed cultivars. Horticulturae 9 (2): 280. https://doi.org/10.3390/horticulturae9020280.

Ermis S, Karslıoglu M, Ozden E, Demir I. 2015. Use of a single radicle emergence count as a vigour test in the prediction of seedling emergence potential of leek seed lots. Seed Sci Technol 43 (2): 308-312. https://doi.org/10.15258/sst.2015.43.2.16.

Ferro JS, Rocha DF, Melo JLAJr, Araújo JC, Neto, Silva J, Pavão JMSJr. 2019. Germination and viability of seeds of *Caesalpinia pulcherrima*, newly harvested and stored. J Agric Sci 11 (7): 73-85. https://doi.org/10.5539/jas.v11n7p73.

Finch-Savage WE, Bassel GW. 2016. Seed vigour and crop establishment: Extending performance beyond adaptation. J Exp Bot 67 (3): 567-591. https://doi.org/10.1093/jxb/erv490.

Guan YJ, Yin MQ, Jia XW, An JY. 2018. Single counts of radicle emergence can be used as a vigour test to predict the seedling emergence potential of wheat. Seed Sci Technol 46 (2): 349-357. https://doi.org/10.15258/sst.2018.46.2.15.

Habyarimana E, Franceschi PD, Ercisli S, Baloch FS, Dall'Agata M. 2020. Genome-wide association study for biomass-related traits in a panel of *Sorghum bicolor* and *S. bicolor* × *S. halepense* populations. Front Plant Sci 11: 551305. https://doi.org/10.3389/fpls.2020.551305.

Hoque MN, Islam MZ, Zohura FT, Mahmud N, Rahman M, Biswas B. 2022. Prediction and standardization of relative emergence and seed vigour in rice (*Oryza sativa* L.) through radicle emergence analysis. J Agric Food Environ 3 (1): 39-44. https://doi.org/10.47440/JAFE.2022.3107.

Human S, Indritama WM, Sihono. 2023. Success of mutation breeding of sorghum to support food security in Indonesia. In: Penna S, Jain SM (eds.). Mutation Breeding for Sustainable Food Production and Climate Resilience. Springer, Singapore. https://doi.org/10.1007/978-981-16-9720-3_14.

Ilbi H, Powell AA, Alan O. 2020. Single radicle emergence counts for predicting the vigour of marigold (*Tagetes* spp.) seed lots. Seed Sci Technol 48 (3): 381-389. https://doi.org/10.15258/sst.2020.48.3.06.

Ilyas S, Widajati E. 2025. Teknik dan Prosedur Pengujian Mutu Benih. IPB Press, Bogor. [Indonesian]

Ilyas S. 2012. Ilmu dan Teknologi Benih: Teori dan Hasil-hasil Penelitian. IPB Press, Bogor. [Indonesian]

International Seed Testing Association (ISTA). 2021. International Rules for Seed Testing. ISTA, Switzerland.

Kamanga BM, Palupi ER, Widajati E, Ilyas S. 2021. Development of a seed vigour testing method using a single count of radicle emergence for true seed of shallot (*Allium ascalonicum* B.). Intl J Sci Food Agric 5 (1): 152-162. https://doi.org/10.26855/ijfsa.2021.03.019.

Khusna AU, Zamzami A, Ilyas S. 2021. Modifikasi suhu uji pemunculan radikula untuk mempersingkat pengujian benih jagung. Indones J Agron 49 (3): 266-272. https://doi.org/10.24831/jai.v49i3.39053. [Indonesian]

Kusumawardana A, Pujiasmanto B, Pardono. 2018. Short communication: Tetrazolium test for evaluating the viability of *Capsicum annum* seeds. Nusantara Biosci 10 (3): 142-145. https://doi.org/10.13057/nusbiosci/n100302.

Lv YY, Wang YR, Powell AA. 2016. Frequent individual counts of radicle emergence and mean just germination time predict seed vigour of *Avena sativa* and *Elymus nutans*. Seed Sci Technol 44 (1): 189-198. https://doi.org/10.15258/sst.2016.44.1.08.

Matthews S, Khajeh-Hosseini M. 2006. Mean germination time as an indicator of emergence performance in the soil of seed lots of maize (*Zea mays*). Seed Sci Technol 34 (2): 339-347. https://doi.org/10.15258/sst.2006.34.2.09.

Matthews S, Powell AA. 2011. Towards automated single counts of radicle emergence to predict seed and seedling vigour. Seed Test Intl 142: 44-48.

Mavi K, Powell AA, Matthews S. 2016. The rate of radicle emergence and leakage of electrolytes provides quick predictions of the percentage of normal seedlings in standard germination tests of radish (*Raphanus sativus*). Seed Sci Technol 44 (2): 393-409. https://doi.org/10.15258/sst.2016.44.2.12.

Mbughi YK, Kilasi NL, Mourice SK. 2024. The effect of seed packaging materials, storage period, and conditions on the physiological sorghum seedlings. J Curr Opin Crop Sci 5 (3): 173-183. https://doi.org/10.62773/jcocs.v5i3.261.

Ministry of Agriculture. 2023. Keputusan Menteri Pertanian Republik Indonesia tentang Petunjuk Teknis Sertifikasi Benih Tanaman Pangan No. 465/ HK220/C/02/2023 [Decree of the Minister of Agriculture of the Republic of Indonesia concerning the Technical Guidelines for Food Crop Seed Certification No. 465/ HK220/C/02/2023]. Ministry of Agriculture, Jakarta. [Indonesian].

Nugraheni, Pujiasmanto B, Samanhudi. 2023. Testing of the rapid vigor method for sorghum (*Sorghum bicolor* L. Moench) by using radicle emergence. IOP Conf Ser Earth Environ Sci 1253 (1): 012108. https://doi.org/10.1088/1755-1315/1253/1/012108.

Ozden E, Memis N, Gokdas Z, Catıkkas E, Demir I. 2020. Seed vigour evaluation of rocket (*Eruca sativa* Mill) seed lots. J Inst Sci Technol 10 (3): 1486-1493. https://doi.org/10.21597/jist.713180.

Ozden E, Ozdamar C, Demir I. 2018. Radicle emergence test estimates predictions of percentage normal seedlings in standard germination tests of aubergine (*Solanum melongena* L.) seed lots. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 46 (1): 177-182. https://doi.org/10.15835/nbha46110871.

Powell AA. 2022. Seed vigour in the 21st century. Seed Sci Technol 50 (2): 45-73. https://doi.org/10.15258/sst.2022.50.1.s.04.

Reed RC, Bradford KJ, Khanday I. 2022. Seed germination and vigor: Ensuring crop sustainability in a changing climate. Heredity 128 (6): 450-459. https://doi.org/10.1038/s41437-022-00497-2.

Sales TS, Júnior VCA, Azevedo AM, Santos MAV, Melo SGF, Nery MC. 2022. Tetrazolium test to assess the viability of kale seeds. J Seed Sci 44: e202244033. https://doi.org/10.1590/ 2317-1545v44261928.

Tao Q, Sun J, Zhang Y, Sun X, Li Z, Zhong S, Sun J. 2022. Single count of radicle emergence and mean germination time estimate seed vigour of Chinese milk vetch (*Astragalus sinicus*). Seed Sci Technol 50 (1): 47-59. https://doi.org/10.15258/sst.2022.50.1.06.

Tonguç M, Guler M, Onder S. 2023. Germination, reserve metabolism, and antioxidant enzyme activities in safflower as affected by seed treatments after accelerated aging. S Afr J Bot 153: 209-218. https://doi.org/10.1016/j.sajb.2022.12.021.

Van der Walt K, Witkowski ETF. 2017. Seed viability, germination, and seedling emergence of the critically endangered stem succulent, *Adenium swazicum*, in South Africa. S Afr J Bot 109: 237-245. https://doi.org/10.1016/j.sajb.2017.01.011.

Wang S, Wu M, Zhong S, Sun J, Mao X, Qiu N, Zhou F. 2023. A rapid and quantitative method for determining seed viability using 2,3,5-triphenyl tetrazolium chloride: With the example of wheat seed. Molecules 28 (19): 6828. https://doi.org/10.3390/molecules28196828.

Wen DX, Hou HC, Meng AJ, Meng J, Xie LY, Zhang CQ. 2018. Rapid evaluation of seed vigor by the absolute content of protein in the seed within the same crop. Sci Rep 8 (1): 5569. https://doi.org/10.1038/s41598-018-23909-y.

Yin MQ, Song WJ, Guo GY, Li F, Sheteiwy MS, Pan RH, Hu J, Guan YJ. 2018. Starchy degradation is related to radicle emergence during wheat seed germination. Seed Sci Technol 46 (2): 359-364. https://doi.org/10.15258/sst.2018.46.2.16.