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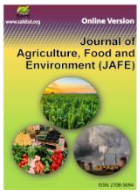

Original Article

Prediction and Standardization of Relative Emergence and Seed Vigour in Rice (*Oryza sativa* L.) Through Radicle Emergence Analysis

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ABSTRACT

An experiment was conducted on fourteen rice varieties to standardize the radicle emergence test for predicting seed germination. Seeds were incubated under a cool white LED's until the final count of germination at 22 ± 2 °C and 82 ± 2 % relative humidity. Radicle emergence and germination were calculated cumulatively and 72 hours was found to be the most optimal time. The high vigor rice varieties germinated in 4 to 5 days, but required repeated or single counts of maximum radicle emergence (MaxRE), which were consistent with normal seedlings obtained at 216 hours with maximum germination (MaxG). Longer lag periods lengthen the mean radicle emergence time (MRET) in several rice varieties. Seed vigour was divided into six categories based on the MaxRE and MaxG values. The MRET and mean germination time (MGT) distinguish vigour in greater detail i.e., five and six groups than the MaxRE and MaxG, respectively. The variance of germination time was substantial for radicle emergence (RE) and germination time. MaxRET (maximum radicle emergence time) and MaxGT (maximum germination time) had the strongest positive significant association (0.997). Mean germination time (MGT) showed highest negative significant correlation (-0.917) with vigour index (VI) and lowest positive significant correlation (0.296) between MaxRE and VI. Whereas MGT showed lowest negative significant correlation (-0.253) with MaxG. The R^2 values between seed vigour parameters and radicle emergence test were significant for MaxRE, MaxRET, MaxG and MGT. Testing and classifying of seed vigour and radicle emergence of rice varieties from different breeding backgrounds or origins or growing seasons and ecotypes, fifty percent radicle emergence time (t_{50RE}) may have the potential to be used alongside mean radicle emergence time (MRET) rather than MRET alone.

Introduction

Rice is one of the major cereal crop of Bangladesh; 'food security' is almost entirely reliant on 'rice security' in Bangladesh, as in most other countries (Brolley, 2015). Rice contributes for around 4.5 percent of GDP in Bangladesh. (BBS, 2020). In agriculture, seed is the most important input. To generate vigorous plants in the field, the quality of seed is very important. Seed vigour is an important factor for crop performance in the field (Qun *et al*., 2007).

In Bangladesh, the formal and informal seed systems provided 35.98 % and 44.33 % rice seed respectively in the years 2013-14 and 2018-19. BADC, one of the largest seed suppliers, supplies the majority of rice seed to meet demand. Challenges like: lack of proper logistic support at BADC for quality seed production and preservation, lack of required amount of breeder seed of desired variety and rapid seed testing facility, are common in quality seed production and supply system intended for the farmers (Amin *et al*., 2020). According to the Asian Development Bank (2012), both quantity and quality seed are in high demand. However, most of the farmers in Asia, including Bangladesh, has a small production area. A large number of seed lots must be analyzed, mandating the adoption of an accurate and rapid quality control approach, particularly to predict seed germination and seed vigor of the seed lots. Seed vigour must be determined in order to forecast a seed lot's planting value. The use of vigour tests by seed producers, such as determining physiological quality, is becoming more common in seed-producing organizations and industries (International Seed Testing Association, 2016).

Seed vigour is a set of qualities that determine seed activity and seed lot performance in a variety of conditions, resulting in satisfactory germination. The vigour test provides a reproducible result that properly indicates the possibility of rapid, uniform emergence in the field, as well as seed lot evaluation (International Seed Testing Association, 2014).

The vigour tests are used to find seed lots that have good storage capacity. In storage and in the field, seed lots with the same germination percentage performed differently (International Seed Testing Association, 2016). Rice seed vigor testing can be done using a variety of approaches. Standard germination tests, accelerated ageing (AA) tests, electrical conductivity (EC) tests, cold tests controlled deterioration testing, and radicle emergence tests are just a few of them. The standard germination test is a rigorous seed quality evaluation that only assesses a seed lot's maximum potential under a variety of environmental conditions. Standard germination testing takes time as well as may not always reveal the full potential of a seed lot, especially in less-than-ideal field conditions (Mavi *et al*., 2016).

Some time-saving vigor classification indirect methods are used, such as electrical conductivity (EC) test, may not be reliable when used on small embryonic seeds. In addition, the accelerated ageing (AA) test is a useful but time-consuming technique (Onwimo *et al*., 2017).

Previous research has identified certain flaws in the methodologies mentioned above which could be resolved by single early count of radicle emergence (Matthews *et al*., 2012b). Radicle (2 mm in length) emergence rate , mean radicle emergence time (MRET), and the time needed for germination of 50 percent viable seed, could be used to assess vigor levels in different species (Mavi *et al*., 2010; Khajeh-Hosseini *et al*., 2010; Matthews *et al*., 2011, 2012a). In 2011, the radicle emergence test for *Zea maize* was accepted as an ISTA-validated vigor test (Matthews and Powell, 2011). Furthermore, single counts of radicle emergence are capable of reflecting deterioration processes in seed in the early germination stage due to metabolism of biochemical components or DNA impairment. The majority of vigor testing procedures (cold test, EC test, and controlled deterioration testing) can produce the same results (Vazquez-Ramos and Sanchez, 2003; Barroco *et al*., 2005; Onwimol *et al*., 2012; Matthews *et al*., 2012b).

Based on single counts of radicle emergence, image analysis could lead to the creation of automated algorithms for creating curves of germination progress and computing germination parameters (Joosen *et al*., 2010). Image analysis will be developed to the point of widespread use in seed industries and research institutes in the not-too-distant future, saving time and providing a more direct method of identifying and counting seeds at the moment of radicle emergence (Belsare and Shah, 2013; Li *et al*., 2015). Therefore, the usage of high-quality seed is required, necessitating quality testing prior to market introduction. Seed vigour tests should be low-cost, quick, simple, objective, and repeatable, with a strong correlation to field performance (International Seed Testing Association, 2016).

Although single counts of radicle emergence and the MRET have been used to explore a range of species, according to Matthews and Powell (2011), nothing is known about rice, particularly rice varieties developed from different genetic backgrounds.

The study's goals were to see if radicle emergence and normal seedling germination curves in rice had any associations, and if a single count of radicle emerging might predict seed vigor in rice. Another interest of this study was to standardize the radicle emergence test so that it could be used to predict emergence in rice grown in various seasons.

Materials and Method

Seed source

In this experiment, samples of fourteen rice varieties (Table 1) were collected from the Bangladesh Rice Research Institute, Regional Station Gopalganj. After harvesting, the seed sample was kept at 25 ± 2°C. Before pre-soaking treatments and germination tests, the seeds were sieved to get only seeds of 3 g of rice seed from each type and manually sorted.

Table 1. Information of 14 rice varieties used in the study.

Rice variety	Season	Special features
BRRIdhan52 (V1)	Aman	Submergence tolerant
BRRIdhan63 (V2)	Boro	Export quality, non-shattering, long grain
BRRIdhan67 (V3)	Boro	Salinity tolerant
BRRIdhan71(V4)	Aman	Drought tolerant
BRRIdhan74 (V5)	Boro	Short duration, Zn enriched rice
BRRIdhan75 (V6)	Aman	25% Less nutrient uptake
BRRIdhan84 (V7)	Boro	Short duration, long flag leaf
BRRIdhan86 (V8)	Boro	Lodging resistant, long grain, export quality
BRRIdhan87 (V9)	Aman	Somaclonal variant
BRRIdhan88 (V10)	Boro	Short duration, suitable for cultivation at haor region
BRRIdhan89 (V11)	Boro	High yielder, short duration
BRRIdhan92 (V12)	Boro	Drought tolerant and derived from rice wheat cross
BRRIdhan96 (V13)	Boro	Lodging resistant, protein rich grain
Bangabandhu dhan100 (V14)	Boro	Zinc enriched, fine grain

BRRI – Bangladesh Rice Research Institute, V - Variety

Germination Test

Laboratory studies were conducted at Bangabandhu Sheikh Mujibur Rahman Science and Technology University's Department of Agriculture and Bangladesh Rice Research Institute's regional station Gopalganj.

The petridish method was used to examine radicle emergence and normal seedlings in three replicates of 100 seeds to minimize the double counting of radicle as root. The seeds were soaked in distilled water at room temperature for 72 hours before being placed on Whatman filter paper no. 1.

Seeds were incubated overnight at 22 ± 2 °C with a relative humidity of 82 ± 2 % using a cold, white light emitting diode as light source. Radicle (2 mm long) emergence and normal rice seedlings were counted at 24 hour intervals till the final count reached 336 hours. Normal seedlings were evaluated according to the International Seed Testing Association's guide on seedling evaluation (2006). Up to 14 days after the test was set, the petridishes were monitored every day and the number of germinated seeds was recorded at 24-hour intervals.

Germination Parameters

Germination

Germination was monitored on a daily basis, and the radicle was considered complete when it protruded nearly 2 mm. The tests were carried out for a total of 14 days (Ellis and Roberts, 1981).

Germination percent

When the seed coat rupture, the plumule and radicle emerged, and they were >2mm long, the seed was termed germinated. The number of germinations was expressed as a percentage. The following formula was used to compute the germination percentage (International Seed Testing Association, 2006):

Germination (%) = (Number of seed germinated/Total number of seed for test) × 100

Speed of germination

The following formula was used to calculate germination speed (Krishnaswamy and Seshu, 1990).

Speed of germination = Number of seed germinated at 72 h / Number of seed germinated at 168h

Vigor index

Daily count of germination of seed was taken to calculate data on vigor index. It was calculated by the following formula (Maguire, 1962). The seed vigor index is the total of all seed qualities and shows the seed's potential level and activity throughout germination and seedling emergence.

Vigor index = No. of germinated seeds(X_1)/ days of first count (X_1) +……+ No. of germinated seeds (X_n)/days at final count (n_n).

Mean germination time (MGT)

Germination was monitored on a daily basis, and the radicle was considered complete when it protruded nearly 2 mm. For maximum germination, the experiment was continued for 14 days (336 hours). The mean germination time (MGT) was calculated using a 9-days (216-hour) count. Mean germination time (MGT) was calculated by using the equation (Ellis and Roberts, 1981):

$MGT=\sum (n\times d)/N$, where n = number of seeds germinated on each day, d = number of days from the beginning of the test, and N = total number of seeds germinated at the termination of the experiment.

Mean radicle emergence time (MRET)

For each replication, the number of seeds that produced a 2 mm long radicle was counted from the start of radicle emergence at twenty-four hour intervals up to 14 days (336 h) (International Seed Testing Association, 2012). Counting up to 9 days (216 h) for mean radicle emergence time (MRET) was examined. The equation was used to compute the mean radicle emergence time (MRET), with some modifications from the formula published by Ellis and Roberts (1981):

$MRET=\sum (n\times d)/N$, where n=number of radicle emerged on each day, d =number of days from the beginning of the test, and N=total number of radicle emerged at the termination of the experiment.

Maximum germination (MaxG, as a percentage) and germination time (t_{50}, in hours) were calculated as germination and radicle emergence indicators, respectively. In all rice varieties, the variance in germination time, the MGT or MRET divided by the t_{50RE} or t_{50G}, is referred to as MGT/t_{50} (Onwimol *et al*. 2016).

Statistical analysis

An analysis of variance was used to examine the data received from various seed germination, radicle emergence, and seedling attributes (ANOVA). Only if the F test of ANOVA for treatments was significant at 0.05 probability levels, the means were separated on the basis of the least significant difference (LSD). Before analysis, values in percent data were arcsine converted. Pearson correlation coefficients and regression were also used to examine the significance of correlation coefficients and regression value using MSTAT-C (Gomez and Gomez, 1984).

Result and Discussion

Different physiological parameters of radicle emergence and germination, viz. Maximum radicle emergence (MaxRE), mean radicle emergence time (MRET), radicle emergence time (t_{50RE}), variance of radicle emergence, maximum germination (MaxG), mean germination time (MGT), germination time (t_{50G}), variance of germination time, speed of germination and vigor index (Table 2) were investigated in rice varieties.

Cumulative radicle emergence and germination parameters

At 22 ± 2°C, the cumulative radicle emergence and germination of each rice variety was evaluated, viz.: V1 = BRRIdhan52; V2 = BRRIdhan63; V3 = BRRIdhan67; V4 = BRRIdhan71; V5 = BRRIdhan74; V6 = BRRI dhan75; V7 = BRRIdhan84; V8 = BRRIdhan86; V9 = BRRIdhan87; V10 = BRRIdhan88; V11 = BRRIdhan89; V12 = BRRIdhan92; V13 = BRRIdhan96; V14 = Bangabandhu dhan100 (Figure 1a, b). The maximum radicle emergence (MaxRE) for the rice seed variety with high vigor was achieved 72 hours after seed germination (HASG) (Figure 1a, b). The MaxRE could classify the seed vigour of rice varieties at this time and sort them as follows: V1 = V2 = V7 = V9 = V10 > V8 > V4 = V14 ≥ V3 = V5 = V11 ≥ V6 > V12 > V13 ($P\leq 0.05$). Rice varieties V1, V2, V7, V9, and V10 had higher seed vigor than V8, whereas V4 and V14 had lower seed vigor than V8. Rice variety V4 and V14 were greater than or equivalent to V3, V5 and V11, and definitely greater than other 3 rice types, viz. V15, V12 and V13. On the other hand V6 exhibited higher vigour than V12 and V13. This was consistent with the results of the analysis of normal seedlings at 216 h (Table 2).

Following the curve-fitting module as also described by Joosen *et al*., (2010) that the germination and cumulative radicle emergence data of rice analyses was comprehensive in the present study. Longer lag periods lengthen the mean radicle emergence time (MRET) in some rice varieties. Furthermore, DNA repair appears to be an early process during the lag phase in several other species as mentioned by Vazquez-Ramos and Sanchez (2003) in different studies with rice seed. As a result, when examining the seed vigour of different rice varieties from different origins or breeding backgrounds, the t_{50RE} may have the potential to be employed alongside MRET rather than MRET alone. When examining both of the MaxRE and MaxG, rice seed vigour might well be divided into six groups (Table 2).

Table 2. Radicle emergence, germination, variance of germination time, speed of germination and vigor index of 14 rice varieties.

Variety	Radicle emergence				Germination				Speed of germination (SOD)	Vigor index (VI)
	MaxE (%)	MRT (h)	T_{50RE} (h)	Variance (MRET/ T_{50RE})	MaxG (%)	MGT (h)	T_{50G} (h)	Variance (MGT/ T_{50G})		
V1	100^{a}	32^{d}	50^{e}	1	100^{a}	50^{f}	43^{d}	1	90	61
V2	100^{a}	31^{d}	40^{gh}	2	100^{a}	41^{f}	23^{gh}	2	90	72
V3	80^{cd}	43^{c}	59^{c}	2	83^{d}	58^{c}	36^{e}	2	57	43
V4	84^{c}	32^{d}	41^{g}	1	92^{b}	54^{d}	37^{e}	1	90	45
V5	80^{cd}	21^{e}	38^{h}	2	76^{e}	27^{g}	13^{i}	2	85	90
V6	76^{d}	45^{c}	41^{gh}	2	84^{d}	44^{f}	20^{h}	2	78	58
V7	100^{a}	53^{b}	56^{d}	1	100^{a}	64^{b}	46^{c}	1	57	45
V8	92^{b}	43^{c}	47^{f}	1	92^{b}	48^{e}	37^{e}	1	74	60
V9	100^{a}	53^{b}	56^{d}	2	100^{a}	62^{b}	37^{e}	2	64	42
V10	100^{a}	43^{c}	54^{d}	2	100^{a}	51de	26^{g}	2	78	48
V11	80^{cd}	53^{b}	74^{b}	2	88^{bc}	61^{bc}	38^{e}	2	62	43
V12	64^{e}	66^{a}	82^{a}	1	68^{e}	88^{a}	68^{a}	1	43	21
V13	48^{f}	57^{b}	59^{c}	1	56^{f}	63^{b}	56^{b}	1	56	23
V14	84^{c}	43^{c}	52^{e}	1	84^{c}	43^{d}	32^{f}	1	77	58

Values are expressed as mean. Mean with different letters within the same row differ significantly at $p \leq 0.05$.
Maximum radicle emergence (MaxRE), mean radicle emergence time (MRET), radicle emergence time (t_{50RE}), variance of radicle emergence, maximum germination (MaxG), mean germination time (MGT), germination time (t_{50G}), Speed of germination (SOD), vigour index (VI) and hours (h).

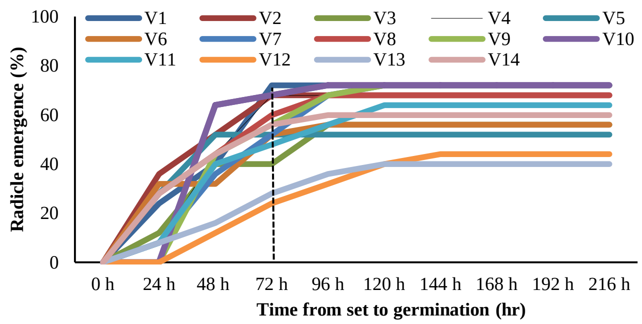

Figure 1 (a) . Cumulative radicle emergence of rice varieties at 22±2°C

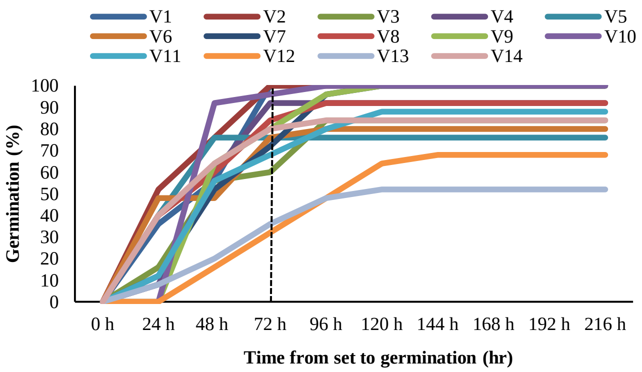

Figure 1 (b). Cumulative germination of rice varieties at 22±2°C

Figure 1. Cumulative radicle emergence and germination in rice varieties: at 22 ± 2°C. (a) represent the arithmetic mean of radicle emergence and cumulative radicle emergence curves of 14 rice variety. (b) arithmetic mean of germination and cumulative germination curves of each variety. The vertical line indicates in (a) and (b) the appropriate time (72 h) for a single count of radicle emergence and final germination count in 14 rice varieties.

On the other hand, MRET and mean germination time (MGT) were able to differentiate vigour in greater depth than that of the MaxRE and MaxG, which included five and six groups, respectively. Because, emergence of radicle and seed germination number were cumulative on the basis of daily count, which is more effective than final count of maximum radicle emergence and maximum germination. The results of classifications using the t_{50RE} and t_{50G} could also be more detailed than those obtained using the MRET and MGT. Which might be used to classify seed vigour into eight categories. The MRET, MGT, t_{50RE}, and t_{50G} were all affected by variations in the varietal group and the amount of seeds per replication in this study, but not the MaxRE or MaxG. However, rice seed varies from varietal group, and as far as we understand, the ratio of an embryonic axis to the whole seed of rice is low, as well as the thick hull for better water absorption. In the present study 72 h has been most appropriate for radicle emergence and germination study because of outcomes of the rice seed vigour classification from MRET and t_{50RE} analysis, at 22°C, took just 4 to 5 d but needed single counts or frequent counts of maximum radicle emergence at 72 h after setting to germinate at 22°C using 100 seeds per replication and which were in agreement with the normal seedlings at 216 h. The results of this study revealed that, in addition to MRET, t_{50RE} was an effective criterion for classifying the seed vigor of different rice varieties grown in different seasons and ecotypes. In this investigation, MRET was successful in vigor classification. MRET was also proven to be successful in classifying the seed vigor of rice as stated by Onwimol *et al.*, (2017) and Chinnasamy *et al.*, (2021), but the results in the earlier findings were based on seed tested from various seed lots of the same rice variety.

Germination time variance

Half of the rice varieties had a germination time variance of 1 (V1, V4, V7, V8, V12, V13, and V14), while the other half had a germination time variance of 2 (V2, V3, V5, V6, V9, V10 and V11). For both Aman and Boro rice cultivars, the variability (MGT/t_{50G}) was the same (Table 2). Variation in the variance of the emergence and germination time (MRET/t_{50}; MGT/t_{50}) in different rice varieties may be caused by differences in growing season and acquired temporary germination attributes (i.e., short dormancy) in rice seed. In Bangladesh there are three rice growing season viz., Aus, Aman and Boro. They varied for photoperiod, growth period and dormancy level. When different varieties are tested in the same time though in control environment there are some difference in seed germination, but this is rarely seen in seeds within the same rice variety in the same or different seed lot, as stated by Al-Mudaris (1998); Ranal and de Santan (2006); Onwimol *et al.*, (2017).

Correlation and regression between and among different parameters of radicle emergence (RE) and germination (G)

To determine the relationship between seed vigour parameters, viz., MaxRE - Maximum radicle emergence (%), MaxRET - Maximum radicle emergence time(h), t_{50RE} - Radicle emergence time (h), MRET - Mean radicle emergence time (h), MaxG - Maximum germination (%), MaxRGT - Maximum germination time(h), t_{50G} - Germination time (h), MGT - Mean germination time (h), SOG - Speed of germination and VI - Vigour index, correlation analysis were used. The range of significant positive correlation values was 0.296 to 0.999, while the range of significant negative correlation values was -0.253 to -0.917 (Table 3). There were 24 negative correlations and 21 positive correlations among the correlations investigated. Among the various seed vigour parameters studied, highly positive significant correlation were observed in MaxRE with MaxG (0.971), MaxRET with MRET (0.997), t_{50RE} with MGT (0.855), MRET with MaxGT (0.997), MaxG with SOG (0.510), MaxGT with MGT (0.859), MGT with t_{50G} (0.904) and SOG with VI (0.789). Onwimol *et al.*, (2017) and Chinnasamy *et al.*, (2021), who reported that mean germination time was highly correlated with time to radicle emergence in rice, support current findings.

Table 3. Correlation coefficients between different parameters of radicle emergence (RE) and germination (G).

	Max RE	Max RET	t_{50RE}	MRET	MaxG	MaxGT	MGT	t_{50G}	SOG	VI
Max RE	0									
Max RET	-0.345*	0								
t_{50RE}	-0.362*	0.833****	0							
MRET	-0.401*	0.997****	0.818****	0						
MaxG	0.971****	-0.281*	-0.327*	-0.335*	0					
MaxGT	-0.351*	0.999****	0.816****	0.997****	-0.282*	0				
MGT	-0.328*	0.871****	0.855****	0.865****	-0.253*	0.859****	0			
t_{50G}	-0.424*	0.749**	0.749**	0.758***	-0.386*	0.731**	0.904****	0		
SOG	0.518*	-0.842****	-0.855****	-0.840****	0.510*	-0.829****	-0.876****	-0.797***	0	
VI	0.296*	-0.855****	-0.761***	-0.870****	0.373*	-0.847****	-0.917****	-0.850****	0.789***	0

*, **, *** and **** significant at $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$ and $P \leq 0.0001$ level of probability with 12 df (n-2)
MaxRE - Maximum radicle emergence (%), MaxRET - Maximum radicle emergence time(h), t_{50RE} - Radicle emergence time (h), MRET - Mean radicle emergence time (h), MaxG - Maximum germination (%), MaxRGT - Maximum germination time(h), t_{50G} - Germination time (h), MGT - Mean germination time (h), SOG - Speed of germination (SOG) and VI - Vigour index.

The relationship between MaxRET and MaxRE (R^2 = 0.1179), MaxGT and MaxG (R^2= 0.0793), MaxG and MGT (R^2 = 0.0642), and MaxRE and MRET (R^2 = 0.161) was investigated using regression analysis. R^2 values in rice varieties were significant in all cases ($P \leq 0.05$). Chinnasamy *et al.*, (2021) reported a similar association between radicle emergence and field emergence in an experiment with rice seed.

These results revealed that there were correlations between radicle emergence and germination test, though the germination test, as determined by normal seedling evaluation, could not completely replace the germination test for all rice varieties. When using the MRET, t_{50RE}, or single counts of maximum radicle emergence for manual counting or image analysis for seed vigor identification, the varietal group and seed size must be taken into account.

Conclusion

Two major goals of this study were to demonstrate the efficacy or feasibility of using t_{50RE}, MRET, or a single count of maximum radicle emergence for different rice varieties at the same time, as well as to establish a low-cost and quick test to classify rice seed vigor. Radicle emergence test and seed germination count were shown to be rapid tests for predicting different levels of vigour for effective plant establishment and classifying rice seed lots or varieties in the current study. Cumulative radicle emergence, germination time determination, and image analysis may be some viable options for dealing with seed vigor categorization and germination test difficulties, it is also extremely probable to construct semi-automatic and fully automated modules using the manual counting method as a reference.

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