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Original Article

Seed vigor classification using analysis of mean radicle emergence time and single counts of radicle emergence in rice (*Oryza sativa* L.) and mung bean (*Vigna radiata* (L.) Wilczek)

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A B S T R A C T

The radicle emergence (RE) test for seed vigor classification is an ingenious protocol that will lead to a fast and reliable automated procedure for verifying seed quality using image analysis. Nevertheless, the success of this protocol has never been described in rice and mung bean that are global staple foods. This experiment analyzed the correlation between RE (2 mm in length) and normal seedlings (NS) during a germination test of rice and mung bean. In total, 12 samples using four cultivars of each species were obtained from different locations and production years. In addition to the germination test, an accelerated ageing (AA) test and an electrical conductivity (EC) test were analyzed. The results revealed that the pattern of the cumulative germination curve of RE and NS coincided but the curve for NS was longer than for RE ($p \leq 0.05$). There was no significant difference in the variance of the germination time between RE and NS of rice but there was a significant difference for mung bean. The vigor levels of the rice seed classified by single counts of RE at 110 h after set to germinate (HASG) conformed to the result of single counts of NS at 200 HASG and the result of the AA test. However, these classifications disagreed with the result derived from the EC test. In contrast, the mung bean vigor level classified by single counts of RE, NS, the AA test and the EC test did not relate well with each other. In conclusion, it is possible to develop the automated procedure for verifying rice seed quality using image analysis *via* a single count of RE.

Introduction

Rice and mung bean are staple foods of Asian people, with the global production of paddy rice and dry bean 2012, being 738 and 24 million t, respectively (FAOSTAT, 2012). Especially for rice, Thailand is one of the world's largest exporters. In 2012, Thailand produced around 37 million t of paddy rice or around 5% of world's production (FAOSTAT, 2012). So, there is no doubt about the parallel demand for the quantity and quality of seed. However, a problem of seed production in Asia is that there is a large number of seed lots to be tested because each farmer has a small production area (Asian Development Bank, 2012), so an accurate and quick method for quality control is needed, particularly to predict the germination and seed vigor of the seed lots.

Previous work on seed vigor testing indicated that an accelerated ageing (AA) test is a useful method for vigor classification in rice but it took approximately 17 d to complete the process (Bradford et al., 1988; Chhetri, 2009). In contrast the electrical conductivity (EC) test is an indirect technique to test vigor and while time-saving, it may not be accurate when applied to small embryonic seeds (Panobianco et al., 2007; Demir et al., 2008). Matthews et al. (2012b) proposed that single early counts of radicle emergence can be used to solve this problem. A quicker comparison of the rate of radicle emergence (2 mm in length), such as the mean radicle emergence time (MRET) and the time required for 50% of viable seeds to emerge (t_{50}) can also predict the vigor level in many species (Khajeh Hosseini, 2009; Khajeh-Hosseini et al., 2010; Mavi et al., 2010; Matthews et al., 2011, 2012a). In addition, there has been some evidence that most vigor testing methods (EC test, cold test and controlled deterioration testing) including single counts of radicle emergence reflected deterioration processes as a result of impairments of metabolism or DNA in the early phase of seed germination (Vázquez-Ramos and de la Plaz Sánchez, 2003;

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Barrôco et al., 2005; Matthews et al., 2012b; Onwimol et al., 2012). The radicle emergence test was accepted into the International Seed Testing Association (ISTA) Rules at the Annual Meeting in Zurich in June 2011 for *Zea mays* as an ISTA-validated vigor test (Matthews and Powell, 2011). This test led to automated methods using image analysis to generate germination progress curves and the calculation of germination parameters from this curve (Joosen et al., 2010). In the near future, the more direct approach of using image analysis to identify and count seeds at the time of radicle emergence will be developed to the point of routine use in research laboratories (Howarth and Stanwood, 1993; Geneve and Kester, 2001; Ducournau et al., 2004; Dell'Aquila, 2005, 2006, 2009; Yazdanbakhsh and Fisahn, 2010; Belsare and Shah, 2013; Li et al., 2015). Although the methods of single counts of radicle emergence and the MRET have been studied in many species (Matthews and Powell, 2011), little is known for rice and mung bean. Therefore, the objectives of this study were: to examine correlations between the germination curves of radicle emergence and normal seedlings in rice and mung bean; to determine the timing of a single count of radicle emergence that is able to predict seed vigor; and to compare the results of radicle emergence and other vigor tests.

Materials and methods

Seed samples

Samples of seven seed lots of rice (*Oryza sativa* L.) were used in this experiment (Table 1). Five samples were obtained from the Rice Department, Ministry of Agriculture and Cooperatives, Thailand, which were produced in northeast Thailand and two samples were derived from a private corporation in central Thailand. All of the samples of mung bean (*Vigna radiata* (L.) Wilczek) used in this experiment were obtained from the Chai Nat Field Crops Research Center, central Thailand (Table 1). Every sample had been stored at 5 ± 3 °C after harvesting. Prior to the experiment, the seeds were sieved to include only seeds of 16–25 mg in rice and 34–40 mg in mung bean using a seed blower machine together with a gravity separator before exposure to aging treatments, germination testing and electrical conductivity testing.

Aging treatments

An accelerated ageing test was used as a seed vigor testing technique to classify the samples of each species. The rice seed was incubated at 44 °C and 100% relative humidity, (RH) for 72 h (Chhetri, 2009) and the mung bean seed was incubated at 45 °C and 100% RH for 96 h (Abass and Shaheed, 2012).

Germination test

The analyses of radicle emergence and normal seedlings were conducted using the top-of-paper method with four replicates of 50 seeds (International Seed Testing Association, 2012), except for lots A4 and B5 where 100 seeds per replicate were used. Seeds were incubated at 25 °C with 24 h illumination using a cool, white light-emitting diode source (Seedburo Achieva Precision Table Top Germinator; Seedburo Equipment Company; Des Plaines, IL, USA). Radicle emergence (2 mm in length and healthy) and normal seedlings were determined at 12 h intervals till 306 and 216 h for rice and mung bean, respectively. Normal seedlings were assessed as described in the handbook on seedling evaluation of the International Seed Testing Association (2006). For germination proficiency testing of analysts, the analysts in this study gave a z-score to normal seedlings less than ±0.2 compared to values obtained from a reference laboratory that had received an A-level for the International Seed Testing Association germination proficiency test.

Electrical conductivity test

To measure seed leachate, bulks of 25 rice and mung bean intact seeds were measured following the conductivity test international rules for seed testing (International Seed Testing Association, 2012). Seeds with a moisture content of 10–14% were submerged in 75 mL of deionized water at room temperature (25 °C) for 24 h. An aliquot of water was removed from each beaker for leachate measurement using a conductivity meter (CyberScan PC 510; Eutech Instruments; Landsmeer, the Netherlands). The results were expressed in microsiemens per centimeter per gram of seed and were calculated following the equation in chapter 15.8.1.7 of International Seed Testing Association (2012).

Data analysis

The GERMINATOR software (Joosen et al., 2010)—a curve-fitting program designed for the analysis of germination data from a four-parameter Hill function by iteration—was used to calculate the germination and radicle emergence indices as maximum germination (MaxG, as a percentage) and germination time (t_{50}, in hours), respectively.

Table 1
Details of rice and mung bean seed samples used in this experiment.

Species	Cultivar	Phenotype description	Harvest time[a]	Seed producer[b]	Production area location	Lot code
O. sativa	RD 6	Short-day plant, sticky and lowland rice	11/2013	RD	15° 15′ 16.30″N, 104° 48′ 16.58″E	A1
O. sativa	RD 31	Lowland rice and less sensitive to photo period.	11/2013	RD	14° 01′ 02.33″N, 100° 44′ 16.69″E	A2
O. sativa	Pathum Thani 1	Lowland rice and less sensitive to photo period.	11/2013	RD	14° 01′ 02.33″N, 100° 44′ 16.69″E	A3
O. sativa	Pathum Thani 1[c]	Lowland rice and less sensitive to photo period.	11/2013	RD	14° 01′ 02.33″N, 100° 44′ 16.69″E	A4
O. sativa	Khao Dawk Mali 105	Short-day plant and lowland rice.	12/2014	RD	15° 15′ 16.30″N, 104° 48′ 16.58″E	A5
O. sativa	Khao Dawk Mali 105	Short-day plant and lowland rice.	11/2013	PC	14° 31′ 01.36″N, 100° 04′ 30.48″E	A6
O. sativa	Khao Dawk Mali 105	Short-day plant and lowland rice.	12/2014	PC	14° 31′ 01.36″N, 100° 04′ 30.48″E	A7
V. radiata	Chai Nat 36	Moderate resistant to powdery mildew	12/2013	CNFCRC	15° 08′ 44.93″N, 100° 11′ 54.04″E	B1
V. radiata	Chai Nat 72	Moderate resistant to powdery mildew	12/2013	CNFCRC	15° 08′ 44.93″N, 100° 11′ 54.04″E	B2
V. radiata	Chai Nat 84-1	Resistance to powdery mildew	12/2013	CNFCRC	15° 08′ 44.93″N, 100° 11′ 54.04″E	B3
V. radiata	Chai Nat 84-1	Resistance to powdery mildew	2/2014	CNFCRC	15° 08′ 44.93″N, 100° 11′ 54.04″E	B4
V. radiata	Chai Nat 84-1[c]	Resistance to powdery mildew	2/2014	CNFCRC	15° 08′ 44.93″N, 100° 11′ 54.04″E	B5
V. radiata	Kamphaengsaen 2	Moderate resistant to powdery mildew	12/2013	CNFCRC	15° 08′ 44.93″N, 100° 11′ 54.04″E	B6

[a] Numbers in column show month/year for example, 11/2013 represents November, 2013.
[b] CNFCRC = Chai Nat Field Crops Research Center; PC = private corporation in central Thailand; RD = Rice Department.
[c] Using 100 seeds per replicate.

Inferential statistics (ANOVA with a single-factor (fixed effect model) and least significant difference post hoc tests) were performed on the results at a significance level of $p \leq 0.05$. The percentage data from the GERMINATOR software, such as MaxG and the Spearman rank correlation coefficient for the fitted curve (r^2), were angularly transformed before the ANOVA was carried out (transformed by arcsine $\sqrt{x/100}$; untransformed values are shown in the table to facilitate comparison). In addition, data dispersal and descriptive statistics were analyzed.

Results

The cumulative radicle emergence and germination determined at 25 °C for each seed lot of rice and mung bean are shown in Fig. 1. For the high vigor seed lots of rice, the maximum radicle emergence (MaxRE) was obtained at 110 HASG on paper (Fig. 1A). At this time point, the MaxRE could classify the seed vigor of rice samples and could be sorted as follows: A5 ≥ A7 = A6 = A1 ≥ A2 > A4 = A3 ($p \leq 0.05$). The seed vigor of A5 was greater than or equal to A7, A6 and A1, and clearly greater than the other three seed lots, of which A2 showed higher vigor than A4 and A3. This result complied with the results of the analysis of normal seedlings at 200 h (Table 2). For the high vigor seed lots of mung bean, 100% radicle emergence was obtained at 40 HASG (Fig. 1). At this time point, the MaxRE could classify the seed vigor of mung bean samples and they could be sorted as follows: B5 = B4 = B6 = B2 = B1 > B3 ($p \leq 0.05$). However, this result was inconsistent when the analysis of normal seedlings was evaluated at 170 h, as some high vigor seed lots reached MaxG (Table 2).

In rice, seed vigor could be categorized into three groups when considering both the MaxRE and MaxG (Table 2). However, the MRET and mean germination time (MGT) could separate vigor in more detail than the MaxRE and MaxG for which four and three groups were shown. Classification using the MRET and MGT could also demonstrate better detail than the results of the classification using the t_{50RE} and t_{50G} which could classify seed vigor into three and two groups, respectively. For mung bean, seed vigor could be classified into three and two groups when considering the MaxRE and MaxG (Table 2). The MGT of mung bean could classify seed vigor into two groups while MRET could not. The best significant

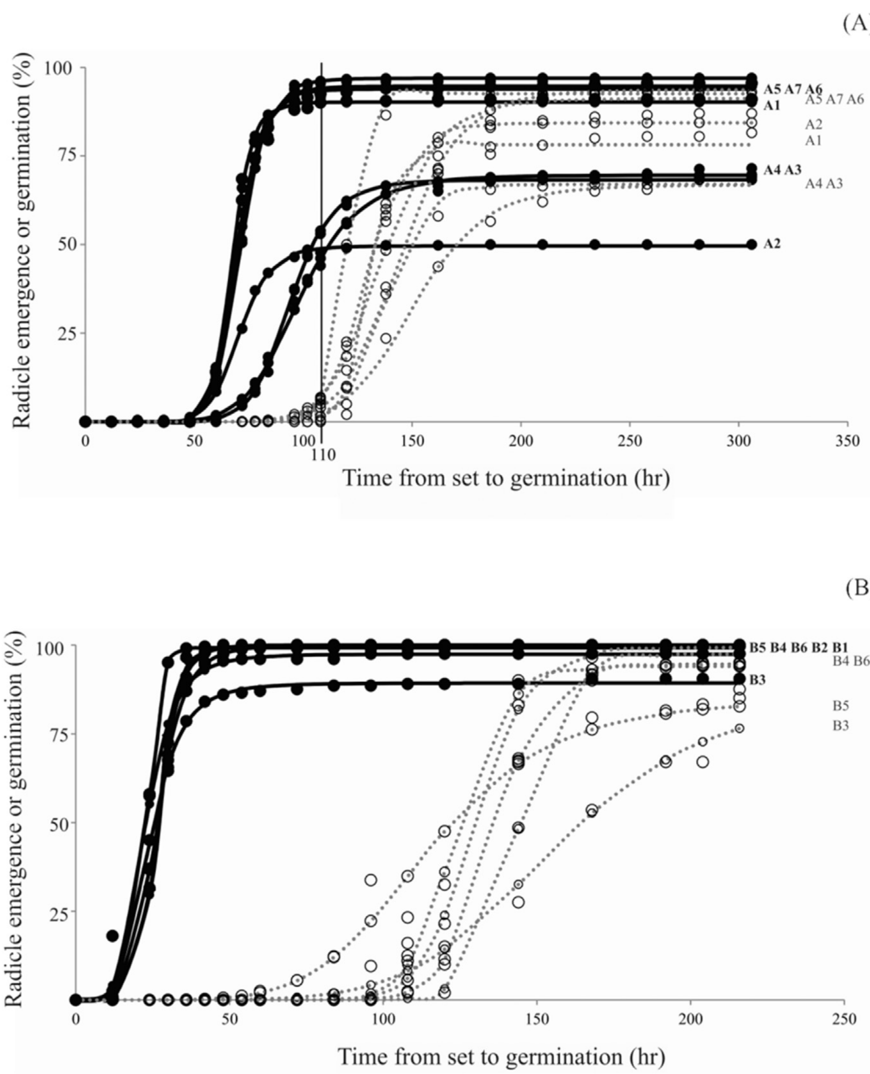

Fig. 1. Cumulative radicle emergence and germination determined at 25 °C for seed lots: (A) rice; (B) mung bean. Closed circles and solid lines represent the arithmetic mean of radicle emergence of each seed lot. Open circles and dotted lines represent the arithmetic mean of germination of each seed lot. Bold letters and gray letters represent the cumulative radicle emergence curves and cumulative germination curves, respectively. The vertical line at 110 h indicates the appropriate time for a single count of radicle emergence in rice.

Table 2
Maximum radicle emergence (MaxRE), maximum germination (MaxG), mean radicle emergence time (MRET), mean germination time (MGT), radicle emergence time (t_{50RE}), germination time (t_{50G}) and Spearman rank correlation coefficient for the fitted curves (r^2) of rice and mung bean seed lots.

Lot code	Radicle emergence			
	MaxRE (%)*	MRET (h)	t_{50RE} (h)	r^2
Oryza sativa				
A1†	95.50[ab]	69.47[cd]	69.18[c]	1.00
A2	91.00[b]	67.13[d]	67.19[c]	1.00
A3	69.00[c]	95.19[b]	93.54[b]	0.99
A4	71.50[c]	101.58[a]	98.50[a]	1.00
A5	100.00[a]	71.68[c]	70.82[c]	1.00
A6	96.00[ab]	70.28[cd]	69.86[c]	1.00
A7	97.00[ab]	71.78[c]	71.25[c]	1.00
Vigna radiata				
B1	99.5[a]	25.99	26.39	1.00
B2	97.5[a]	23.55	23.06	1.00
B3	90.5[b]	25.04	24.23	1.00
B4	100.0[a]	26.62	26.78	1.00
B5	100.0[a]	23.16	24.51	0.98
B6	99.5[a]	21.73	22.86	1.00
Lot codes	**Germination MaxG (%)**	**MGT (h)**	**t_{50G} (h)**	**r^2**
Oryza sativa				
A1	81.50[c]	131.57[bc]	130.62[bc]	1.00
A2	87.00[bc]	132.98[bc]	131.56[bc]	1.00
A3	69.00[d]	139.27[abc]	137.99[abc]	1.00
A4	69.80[d]	154.65[a]	152.07[a]	0.99
A5	95.00[a]	148.65[ab]	146.64[ab]	1.00
A6	93.00[ab]	142.23[ab]	140.91[ab]	1.00
A7	95.50[a]	120.39[c]	119.74[c]	1.00
Vigna radiata				
B1	95.00[a]	137.80[ab]	136.06[c]	1.00
B2	94.00[ab]	125.29[cd]	123.65[d]	1.00
B3	87.50[c]	131.88[bc]	156.80[a]	0.98
B4	99.50[a]	131.39[bc]	129.69[cd]	1.00
B5	88.60[bc]	118.60[d]	114.12[e]	0.96
B6	97.50[a]	147.26[a]	145.07[b]	1.00

* Values in columns in each section within each section followed by different lowercase superscript letters are significantly different ($p < 0.05$).
†A1 = RD 6; A2 = RD 31; A3 = Pathum Thani 1; A4-A7 = Khao Dawk Mali 105; B1 = Chai Nat 36; B2 = Chai Nat 72; B3–B4 = Chai Nat 84-1; B5 = Kamphaengsaen 2.

difference of mung bean seed vigor was found when using the t_{50G} which could be separated into four groups. There were no significant differences among the Spearman rank correlation coefficients for the fitted curve using the four-parameter Hill function for every lot (Table 2).

The results of the vigor classification from the MRET related with the results from AA test ($r = -0.926$, $p \leq 0.001$) as shown in Table 3. The AA test is a method commonly used in the classification of seed vigor in rice, but it requires more equipment and time (up to 336 h) as well as being more laborious (Chhetri, 2009). Correlation coefficients between the results of the AA test and the EC test were low ($r = 0.18$ to 0.35, $p \leq 0.13$). In the same manner, the correlation coefficients between the MRET and MGT including the AA and EC tests of mung bean were also low (Table 3).

Table 3
Significance of correlation coefficients between mean radicle emergence time (MRET) and mean germination time (MGT), accelerated ageing (AA) test (%) and electrical conductivity (EC) test.

MRET	MGT	AA	EC
Rice	0.435[a]	−0.926[b]	−0.29
Mung bean	−0.054	−0.124	0.167

[a] $p < 0.05$.
[b] $p < 0.01$.

These results revealed that there were relationships between radicle emergence and the germination test ($r = 0.89$, $p \leq 0.001$), although the germination test, by normal seedling evaluation, could not be replaced by the analysis of radicle emergence. In Fig. 2, the variance of germination time, the MGT or MRET divided by the t_{50RE} or t_{50G}—the so-called MGT/t_{50}—in all rice samples were close to 1 and no statistically significant differences were found. The variability of MGT/t_{50} in mung bean was greater than in rice, especially for B6, where there were statistically significant differences in MGT/t_{50} (Fig. 2).

Discussion

Seed vigor identification and germination testing are very important for crop production because these allow farmers to implement their production plan more easily. However, such actions are laborious and require highly experienced workers (Dell'Aquila, 2005; Belsare and Shah, 2013). Consequently, seed managers are trying to develop and standardize for convenience and economy in standard germination and seed vigor testing (Asbrouck and Taridno, 2009; Matthews et al., 2012b; Bradford et al., 2013). In seed vigor classification and germination, normal seedling testing using image analysis may be one potential way to deal with such problems (Howarth and Stanwood, 1993; Belsare and Shah, 2013). However, the analysis of normal seedlings is subjective and is difficult to evaluate. Therefore, seed managers are trying to use radicle emergence instead of dealing with the normal seedling because it is less subjective (Geneve and Kester, 2001; Ducournau et al., 2004; Dell'Aquila, 2005, 2006, 2009; Yazdanbakhsh and Fisahn, 2010; Li et al., 2015). The present results showed that the MRET was a good parameter to classify the seed vigor of rice, whereas the t_{50RE} might be used in mung bean. The MRET has been successful in vigor classification in many species such as maize (Khajeh Hosseini, 2009; Matthews et al., 2011), cabbage (Demir et al., 2008), oilseed rape (Khajeh-Hosseini et al., 2010; Matthews et al., 2012a), watermelon, melon and cucumber (Mavi et al., 2010). The results in the present experiment repeated this success with rice but not with mung bean. It might be possible that the structural details of normal seedlings of mung bean, with its dicotyledonous angiosperm clade, is more difficult to evaluate and consequently is more subjective than in rice, which has a monocotyledonous angiosperm clade (ISTA, 2006). This phenomenon also might result in fluctuation in the variance of the germination time (MGT/t_{50}) in mung bean but is rarely displayed in rice (Al-Mudaris, 1998; Ranal and de Santana, 2006). It was noteworthy that the difference between the skewness of radicle emergence and normal seedlings of rice did not exceed 0.5 in every sample, while in most of the mung bean samples, it exceeded 0.5 (data not shown). The results of vigor classification from the MRET were related with the results from the AA test which were related to the field emergence test (Chhetri, 2009). However, the AA test is laborious and requires more than 300 h to complete. In addition, it is evident that DNA repair is an early event during the lag period in several other species (Vázquez-Ramos and de la Plaz Sánchez, 2003). The longer the lag period, the higher the MRET, and in the seed lots of cucurbits and cabbage this can be explained in terms of aged seed needing a longer time for repair (Demir et al., 2008; Mavi et al., 2010). So, the MRET may have the potential to be used instead of AA testing in rice. However, much remains to be learned about metabolic repair in such a period for these two species, especially in mung bean.

In the present experiment, the GERMINATOR curve-fitting module was able to provide analysis in both the cumulative radicle emergence and germination data of rice and mung bean. This revealed the efficiency of this module as also reported by

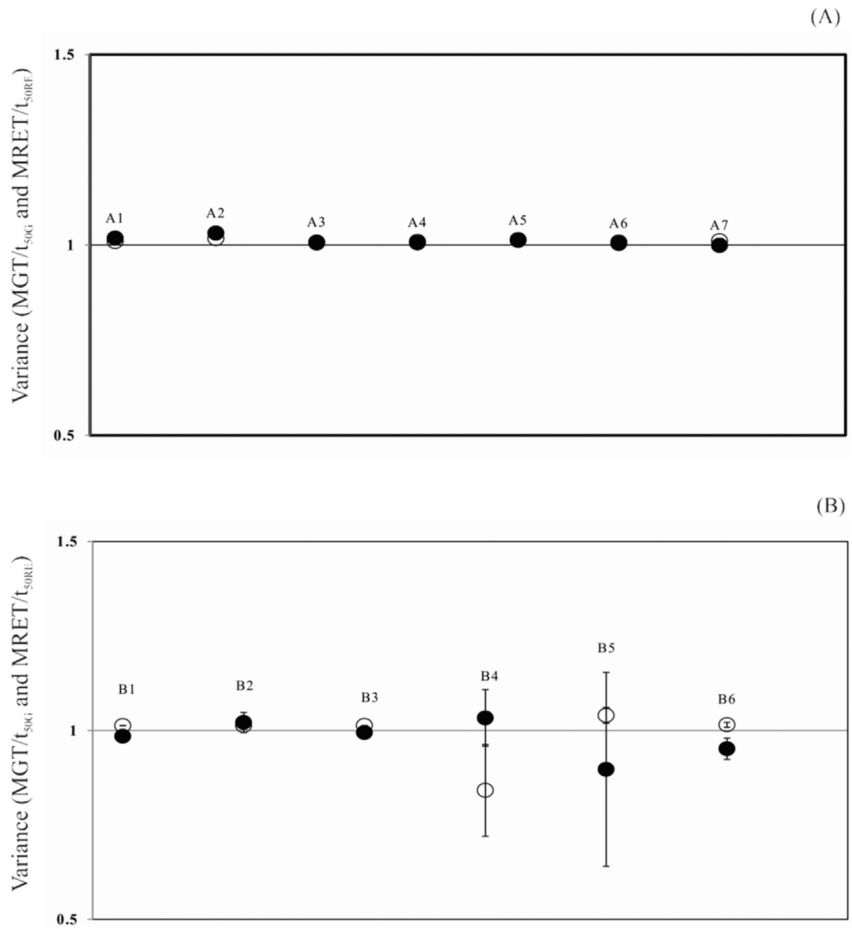

Fig. 2. Variance of germination time, mean germination time (MGT) or mean radicle emergence time (MRET) divided by time required for 50% of viable seeds to emergence or germinate (t_{50RE} or t_{50G}), of each rice (A) and mung bean (B) sample. The closed circles represent the arithmetic mean of radicle emergence and open circles represent the arithmetic mean of germination of each seed lot. Error bars denote the confidence intervals error bars ($n = 4$; $p < 0.05$); missing error bars indicate that they are smaller than the symbols.

Joosen et al. (2010). The present study also identified a difference between the number of seeds per replicate affecting the MRET and t_{50RE}. The MRET was affected by the number of seeds per replicate but this effect was not found with the MGT. However, this suggests that the seed number per replicate needs to be taken into consideration when seed vigor identification uses the MRET or single counts of maximum radicle emergence for image analysis. The correlation coefficient between the AA test and EC test was low in this study suggesting that the vigor classification using the EC test with the 24 h soaking period may be improper. This may be attributable to the fact that the ratio of the embryonic axis to the whole seed of rice is low and also to the thick hull (Panobianco et al., 2007; Demir et al., 2008). The result was high variability and a low value in the electrical conductivity test (Powell and Matthews, 2005). One of the objectives of this work was also to develop a low-cost and quick test to classify the seed vigor of rice and mung bean that allowed monitoring of several hundreds of germination and vigor tests by a single person. In conclusion, the results of the rice seed vigor classification from the MRET analysis, at 25 °C, took only 4–5 d but required frequent counts or single counts of maximum radicle emergence at 110 h after setting to germinate on paper, at 25 °C and using 100 seeds per replicate which were in accordance with the results from the analysis of normal seedlings at 200 h and also with the AA test. Therefore, it is highly possible to develop an automated module using image analysis of radicle emergence to classify the seed vigor of rice cultivars in Thailand.

Conflict of interest

There is no conflict of interest.

Acknowledgements

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