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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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ABSTRACT

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.