An Automatic Method for Rice Seed Vigor Classification Via Radicle Emergence Testing Using Image-Processing, Curve-Fitting and Clustering Methods

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Research

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Abstract

Background: Rice seed vigor classification is important for seed storage management by seed producers and by farmers while planning their cultivation activities. Field emergence is a direct method of seed vigor testing but is laborious, time-consuming and subjective. The accelerated aging (AA) test is often used as an indirect method for rice seed vigor classification in the laboratory. However, the results from this method are often imprecise. This paper presents the SVRice package, a simple, cost-efficient and flexible procedure that utilizes computer image analysis for high-throughput, automatic rice seed vigor classification. SVRice consists of 4 steps: dynamic imaging, image processing, curve fitting and clustering. Seed vigor was classified based on radicle emergence indices, such as maximum radicle emergence (MaxRE), mean radicle emergence time (MRET), radicle emergence speed (t_{50}), uniformity of radicle emergence (U_{7525}), and area under the curve of the radicle emergence fitted curve (AUC).

Results: Parameters used to classify rice seed vigor, such as MRET, U_{7525} and t_{50}, were strongly negatively correlated with the saturated salt accelerated aging (SSAA) test. A germination time of 90 hours at 25°C was sufficient for effective classification based on SVRice, whereas the SSAA test took approximately 400 hours to complete. The SVRice software algorithm was created to be especially suitable for assessment after 6 months under controlled atmosphere storage (at 15°C and 37% RH in a hermetic bag). The study showed that SVRice could unambiguously classify 40 indica rice samples with different varieties, production years, production sites, storage times and storage conditions compared with the SSAA test.

Conclusions: This paper confirmed the accuracy, reproducibility and flexibility of the SVRice package for automatic seed vigor classification of *Oryza sativa* seeds; moreover, it is also likely applicable to other species as a viable alternative to current methods that require more time and are less precise.

Background

Seed vigor is an outstanding and important characteristic for seed producers in quality seed production. For farmers, seed vigor is often considered for planning in plant production. Seed vigor reflects germination in a wide range of environments and/or the storability of seed lots [1, 2]. In addition, seeds with high vigor must have a high germination percentage in the laboratory. However, seeds with a high germination percentage may not necessarily have high vigor [3].

Seed vigor testing of a seed lot may use either a direct or indirect method under standardized conditions. The methods are often conducted using procedures that are laborious, time-consuming and subjective. Field emergence is a direct method of seed vigor testing. Indirect tests are faster than direct methods but still take approximately one week, depending on the species, and are still highly subjective; for example, the accelerated aging test and evaluation of seedling shoot length [1, 4, 5]. Currently, the radicle emergence test—a fast and reliable method to evaluate seed vigor—has been applied in many species [6–9]. The major advantages of this method are convenience and precision. There is also a high possibility of developing an automatic or semiautomatic method for seed vigor testing using image analysis or machine vision through a radicle emergence test [10–12].

Seed vigor testing using image analysis has been developed in many ways, depending on the different materials of the test, such as analysis of dyed seeds with tetrazolium or seedling growth [13–18]. However, the seeds must be prepared using a chemical-dyeing process or analyzed using a complex system, such as a hyperspectral camera or the use of a micro-optrode technique before image analysis [19–23]. Interestingly, there have been no published reports on a method to integrate radicle emergence testing and image analysis for rice seed vigor classification, although a variety of seed quality phenotyping platforms have been used with many crops [24–26]. Such a new process would not require a long germination period or chemicals but rather would only require frequent photographs (2 mm radicle stage) during seed germination.

Rice is the third most popular food grain after corn and wheat [27]. Indica rice seeds take approximately 3–5 days for the radicle to emerge, depending on geographical race [28, 29]. It is different from germination in normal seedlings, in which the test period may be extended up to 14 days [1]. Therefore, vigor classification of indica rice using the radicle emergence test with image analysis is of interest as a possible approach. This process would help seed producers recognize the vigor of the rice seed quickly and precisely through an automated system.

This research aimed to automate seed vigor testing using a software system incorporating image analysis called SVRice that could unambiguously classify many varieties of rice seeds from various production sites and different storage conditions and storage times. SVRice software was designed for rice seed vigor classification of radicle emergence data using a four-parameter Hill function to process and calculate germination indices, such as maximum radicle emergence (MaxRE, %), mean radicle emergence times (MRET, hours), radicle emergence speed (t_{50}, hours), uniformity of radicle emergence (U_{7525}, hours) and area under the curve of the radicle emergence fitted curve (AUC) of the radicle emergence fitted curve (Fig. 8ˆ). The SVRice package consists of two important parts: analysis of radicle emergence using image analysis based on an 8-step image segmentation technique and calculation of t_{50}, MRET and U_{7525}. Then, the sample can be classified based on the calculated radicle emergence parameters using cluster analysis. The results from SVRice can be compared with the results of visual assessment and standard methods for seed vigor testing. This research will reveal the possibilities of rice seed vigor evaluation using a method that integrates the radicle emergence test and image analysis.

Results

Seed germination

Germination tests were evaluated using the top-of-paper technique with four replicates each with 100 seeds [1]. The percentage germination of rice seeds stored under ambient conditions (on the laboratory bench) was less than that under the controlled conditions over time, especially after 12 months of storage (Fig. 1). Storage of the indica rice seed under ambient conditions at higher humidity and temperature reduced germination more than storage under controlled conditions at 15°C and 37% RH in hermetic GrainPro® bags (Fig. 1a).

The germination of glutinous rice, code G, was greatly reduced compared with that of the other varieties. After 12 months of storage, rice variety RD6 stored under ambient conditions ceased to germinate (Fig. 1b).

Seed vigor classification via saturated salt accelerated aging—SSAA—test

The SSAA test was used as a seed vigor test. The SSAA test is a well-known seed vigor test in many species [1]. The test assesses the response of seeds through germination tests after they have been subjected to high temperature and relative humidity for a particular period of exposure [30, 31]. The SSAA value (%) represented resistance to hot and humid conditions by the seed. The controlled conditions not only maintained germination of rice seeds but also delayed a reduction in seed vigor for 6 months (Fig. 2a). However, storage under ambient conditions decreased seed vigor rapidly (Fig. 2b). Interestingly, the KDML105 rice seed produced in Khon Kaen (code D) could maintain seed vigor, even under ambient conditions.

Seed vigor classification using the SVRice package

The SVRice package consists of 4 important steps, namely, imaging, image processing, curve fitting and clustering. After curve fitting, radicle emergence indices, such as MaxRE, MRET, t_{50}, U_{7525} and AUC, were calculated and used to classify rice seed vigor using a single index of radicle emergence via analysis of variance (ANOVA) at a significance level of $p \leq 0.05$, followed by post hoc tests with Tukey's honest significant difference (Tukey's HSD). For example, rice seeds after 12 months of storage under controlled atmospheric conditions were classified into 2, 3 and 2 groups using MaxRE, MRET and t_{50}, respectively, while seeds after storage under ambient conditions for 12 months were classified into 5, 2 and 3 groups using the same parameters (Table 1). The results varied according to the radicle emergence index used.

Table 1
Radicle emergence indices for various varieties of indica rice seeds after 12 months of storage under different conditions using an image-processing assay in SVRice

Code	Controlled atmosphere storage*					Ambient storage†				
	Maximum radicle emergence (%)	Mean radicle emergence time (hours)	Radicle emergence speed (hours)	Uniformity of radicle emergence (hours)	Area under the curve of the radicle emergence fitted curve‡	Maximum radicle emergence	Mean radicle emergence time (hours)	Radicle emergence speed (hours)	Uniformity of radicle emergence (hours)	Area under the curve of the radicle emergence fitted curve
A	98.0 ab§	76.3 cd	74.9 ab	16.6 c	206.6 ab	72.3 c	173.7 abc	203.8 a	108.3 a	66.0 de
B	97.0 ab	96.6 ab	88.4 a	53.4 ab	191.9 cb	48.0 d	170.4 abc	195.9 a	105.3 a	48.5 e
C	99.3 ab	84.3 cb	79.4 a	34.3 bc	208.2 ab	98.8 a	117.8 cd	112.0bc	48.9 bc	177.2 b
D	99.5 ab	82.9 bcd	77.1 ab	36.7 abc	208.5 ab	98.8 ab	95.8 d	89.2 bc	44.4 bc	200.3 ab
E	95.5 ab	82.4 bcd	78.2 ab	30.9 bc	195.8 b	75.0 c	178.0 ab	198.4 a	97.8 ab	72.8 d
F	100.0 a	63.9 d	61.0 ab	22.1 c	230.2 a	98.8 a	77.0 d	71.5 c	34.6 c	217.7 a
G	94.3 b	109.9 a	102.2 ab	65.6 a	166.6 c	4.5 e	171.5 a	214.4 a	113.6 a	4.3 f
H	100.0 a	85.1 cb	77.7 b	54.7 ab	202.7 b	93.5 b	129.8 bcd	133.4 b	96.6 ab	143.1 c
Pr > F	0.0060	< .0001	< .0001	0.0002	< .0001	< .0001	< .0001	< .0001	< .0001	< .0001
CV (%)	5.701	9.588	8.659	33.413	5.650	5.960	17.639	15.493	28.066	8.550

* Seeds stored at 15°C and 37% RH

† Seeds stored at 29°C and 53% RH

‡ Area under the curve is the integration of the fitted curve between t = 0 and 300 hours

§ Means within a column of each factor with the same lowercase letters are not significant at $p \leq 0.05$ based on Tukey's honest significant difference test

Generally, the SVRice package provided a systematic analysis of results, but it was slightly different from visual analysis (see Supplementary material 1). Differences between the detection of radicle emergence based on visual analysis versus the SVRice package were analyzed using a box plot (Fig. 3)

that showed that there was an increase according to the germination time. Rice seeds were dispersed on steel blue seed germination blotter paper using a mask to ensure accurate and reproducible spacing. Therefore, clustering of seeds was prevented as much as possible. However, after 120 hours, rice seedlings touched each other, which caused clustering, resulting in greater differences between the visual analysis and SVRice software. Interestingly, the SVRice package was especially optimized with indica rice seeds stored under a controlled atmosphere at 6 months (Fig. 3b). Nevertheless, the image processing in the SVRice package was effective when used with samples stored under ambient conditions for 12 months due to their low percentage radicle emergence (Fig. 3c).

The image-processing algorithm of the SVRice package was effective in evaluating seed vigor for indica rice grown in Thailand, although radicle emergence behavior differed markedly according to the conditions and storage time (Fig. 4). At 96 hours after the start of imbibition, the percentage of radicle emergence of RD6 (Fig. 4e) was less than that of KDML105 at the same time (Fig. 4b). The seed vigor of glutinous rice (code G) was lower than that of another variety (code A) because it had higher values for MRET, t_{50} and U_{7525} but lower values for MaxRE and AUC.

The SSAA test was correlated with the individual seed parameters derived from the image processing and radicle emergence, curve-fitting module of the SVRice package (Fig. 5). Negative linear correlations were observed, with a high SSAA test result correlating with low values for MRET, t_{50} and U_{7525} and vice versa ($p \leq 0.05$). Therefore, the classification of rice seed vigor using their individual parameters derived from the image processing and radicle emergence, curve-fitting module of the SVRice package was likely to reflect the SSAA test. However, the use of multiple radicle emergence parameters together from the SVRice package could give more precise and accurate results. K-means clustering was used to partition the rice seed samples into K clusters in which each observation belonged to the cluster with the nearest mean using all radicle emergence parameters.

After clustering using the SVRice package, all indica rice seed samples used in the experiment were classified into 2 clusters: high and low seed vigor (Fig. 6e). The optimal number of clusters could be adjusted as needed, although the appropriate number was recommended according to the silhouette method. Figure 6d illustrates a K-means cluster diagram for the 40 rice seed samples used in the experiment, which differed in genetics, production time, storage time and storage condition. At 6 and 12 months of storage, old seed and glutinous rice seeds were classified as having low vigor (Fig. 6a–c). Generally, the vigor of rice seeds stored under controlled conditions was higher than that under ambient conditions; the results for each sample are detailed in Supplementary material 2.

The SVRice clearly classified KDML105 rice seeds from different production years, even though it could not classify the samples from the different production areas (Fig. 7). KDML105 rice seeds produced in 2017 were clearly distinguished from those produced in 2019 (Fig. 7c), although there was no apparent difference in the seed coat based on observations with the naked eye (data not shown).

Discussion

Seed vigor is a notable characteristic that allows efficient pre- and postharvest management by seed scientists. As a field emergence test, the direct seed vigor test is laborious and resource-consuming [1, 8]. In addition, the results are subjective, as they are heavily dependent on the expertise of the tester. Therefore, seed scientists have been attempting to find a convenient, economical and standardized method for seed vigor testing [32]. Unfortunately, to date, there has been no report of a universally acceptable single indirect test for assessing seed vigor [7]. For this reason, multiple tests should be used together to make the best assessment. The indirect methods commonly used produce one parameter per test. Therefore, an indirect method of seed vigor testing requires more than one method to confirm the results. In the past, seed scientists have used two or three laboratory-based indirect methods together to accurately determine seed vigor, including the stress test, electrical conductivity test and seed growth rate test [2, 31, 33]. Other methods, such as fast ethanol assays, do not consider seedling performance but rely on expensive sensors and have not been reported to be successful with rice seeds [34, 35].

Recently, the radicle emergence test has been recognized as being effective and fast for seed vigor testing, as supported by the metabolic repair hypothesis [7]. This hypothesis provides the overall physiological basis to explain the principles behind the standard germination and vigor tests, but the radicle emergence test is sensitive and can vary according to species and test conditions [9, 28, 36]. Interestingly, Joosen, et al. [37] offered GERMINATOR software that can calculate more than one parameter in a single germination test with a cumulative germination curve through the four-parameter Hill function. Furthermore, it is possible to classify seed vigor automatically using machine vision combining the concepts of the GERMINATOR software with image processing and cluster analysis as revealed in the SVRice package. After imaging (image or video), the information on radicle emergence for each seed at a particular time is processed and plotted as a cumulative radicle emergence curve based on the Hill function. This allows the radicle emergence indices to be calculated for multiple parameters (MaxRE, MRET, t_{50}, U_{7525} and AUC). The MRET, U_{7525} and t_{50} values are of particular importance because they had a strong negative correlation with the SSAA test (Fig. 5), and they could accurately determine rice seed vigor after cluster analysis. The SSAA test classified seed vigor into 2 groups using ANOVA and a post hoc test (Fig. 2). However, the analytical results were questionable due to entanglements in the classification. In contrast, the results from SVRice could be clearly separated using the five radicle emergence indices (MaxRE, MRET, t_{50}, U_{7525} and AUC), as shown in Fig. 6e. SVRice successfully differentiated samples from the same variety but in different production years. The number of clusters was reliable because relative clustering validation was used to determine the optimal number of clusters and evaluated the clustering structure using varying parameters for the same algorithm (varying the number of k clusters) [38]. In comparison with high-end seed phenotyping devices such as the SeedGerm system [24], our image-processing algorithm and hardware design follow a specific-use and easy-to-build strategy. Unfortunately, we cannot compare the results of SVRice with other germination phenotyping platforms using the same images because their commercial versions are not made for rice seeds.

A germination time of 90 hours at 25°C was sufficient for effective classification based on SVRice, whereas the SSAA test took approximately 400 hours to complete. These results were consistent with Onwimol, et al. [29], who proposed a single count of radicle emergence at 110 hours after setting to germination at 25°C for the identification of the vigor of rice seeds. In the current experiment, especially under ambient storage, the radicle emergence behavior of rice seeds was very different for rice seed samples covering the popular rice varieties in Thailand (Fig. 4). The SVRice software algorithm was set up to be especially suitable for assessment after 6 months under controlled atmosphere storage because this storage condition and period are common for rice production in Thailand (Fig. 3b).

Our image-processing algorithm can be easily adapted to desired varieties or radicle emergence behavior through the adjustment of the number of groups in Otsu's multiple thresholding in the seed segmentation step and the structuring element for morphological dilation in the counting step. For calibration, the results after adjustment of the algorithm can be compared with the results from visual analysis—manual scoring—that can be used as a reference. Uncontrolled illumination in the image acquisition step might result in failure to obtain a result in the seed segmentation step. Otsu's multiple thresholding might group some parts of the seed into the background. Long, messy overlapping or touching roots, as shown in Fig. 4c, might result in an incorrect number of seeds in the counting step. The counted seed number might be greater than the number of seeds because of substantial variations caused by too many overlapping radicles at the late germination stage. Due to the potential of the K-means, the limitation of SVRice is the classification ability of the package that can be classified up to the grouping level but cannot be classified to a single sample level [39]. However, it should be apparent that the classification of seed vigor up to a single sample level does not provide much information to seed scientists to make decisions during seed inventory management, which, if necessary, can be solved by using an appropriately trained protocol with reference to an authentic dataset of the in-house protocol.

The developed software is suitable for high-throughput seed vigor classification. The SVRice package utilizes dynamic imaging to compare the image to that from previous time points. Hence, it can detect changes in radicle emergence better than using single end-point imaging analysis. Consequently, the results are more accurate, and less time is required. SVRice software can be applied to detect seed health and seed damage from insects during storage in a warehouse if multispectral imaging is utilized [40]. The SVRice pipeline offers a well-defined and robust experimental setup but is flexible in terms of numbers and treatments. Improved efficiency and the absence of subjectivity are great advantages in computer-aided assessment. Automatic seed vigor classification was optimized for indica rice and would most likely work for many other species as well.

Conclusions

A design system utilizing an algorithm with dynamic imaging, image processing, curve fitting and clustering enables classification of seed vigor that can be adapted for all plant species. The package can be optimized to set accurate thresholds by comparing the automated scoring with manual scoring. In conclusion, the SVRice package is a low-cost package that allows the monitoring of several hundred seed vigor tests by a single person.

Materials And Methods

Seed sources

This experiment used five indica rice varieties with different harvest times, seed producers and production areas (varieties A–H), as shown in Table 2. After harvesting, the seeds were stored at 15 ± 1°C and 50 ± 5% relative humidity (RH) until use in this experiment. Before the experiment began, the seeds were graded to include only those that weighed 200 ± 100 mg and were stored at 15 ± 1°C in darkness prior to the experiment.

Table 2
Details of the indica rice seed samples used in this experiment

Code	Variety	Phenotype description	Harvest time*	Seed producer	Production area location
A	KDML105	Nonglutinous, photoperiodic, low-amylose*, fragrant	2019	Government agency	15° 19' 23.1424" N, 104° 42' 59.8156" E
B	KDML105	Nonglutinous, photoperiodic, low-amylose, fragrant	2017	Private corporation	14° 38' 56.2963" N, 100° 0' 1.5466" E
C	KDML105	Nonglutinous, photoperiodic, low-amylose, fragrant	2019	Government agency	14° 1' 0.7532" N, 100° 43' 37.4297" E
D	KDML105	Nonglutinous, photoperiodic, low-amylose, fragrant	2019	Government agency	14° 32' 5.5464"N, 102° 7' 3.7333"E
E	RD31	Nonglutinous, day-neutral plant, high-amylose†	2019	Government agency	14° 32' 5.5464"N, 102° 7' 3.7333"E
F	PTT1	Nonglutinous, day-neutral plant, low-amylose, fragrant	2019	Government agency	15° 14' 51.8485"N, 100° 5' 37.9198"E
G	RD6	Glutinous, photoperiodic	2019	Government agency	15° 19' 23.1424" N, 104° 42' 59.8156" E
H	SYP	Nonglutinous, photoperiodic, low-amylose, red-pericarp	2019	Government agency	7° 33' 53.9842"N, 100° 7' 20.6152"E

*Amylose content < 12%, † Amylose content > 22%.

Each sample was divided into approximately equal halves, with one stored in a special plastic bag (SGB Premium-25RZ GrainPro® SuperGrainbag®, GrainPro, Zambales, the Philippines) and the other stored in a standard clear polyethylene (PE) bag. The special bag, SGB Premium-25RZ GrainPro® SuperGrainbag® (GrainPro, Zambales), was stored in a controlled atmosphere (at 15°C and 37%RH), while the PE bag was placed on a laboratory bench under ambient conditions (approximately 29°C and 53%RH), based on monitoring using a USB data logger (Centor Thai, Bangkok, see also Supplementary material 3). At 6 months and 12 months of storage, samples were taken from each bag and subjected to germination and vigor tests.

Germination test

Germination tests were evaluated using the top-of-paper technique [1] with four replicates each with 100 seeds. Seeds were germinated on the top of two layers of steel blue seed germination blotter paper (Anchor®, Minnesota), which were placed in a polystyrene transparent box (9.2 × 28 × 5.7 cm^3), which was then closed and placed in a cabinet germinator (Seedburo Equipment, Illinois). The RH in the germinator was maintained at very near saturation, and the temperature was set to 25°C. Seed radicle emergence (2 mm radicle) and germination were tested. Seeds were scored as germinated when normal seedlings were visible according to ISTA [41]. Radicle emergence and normal seedlings were counted daily until 14 days after setting to germination. Determination of the speed and determination of the uniformity of radicle emergence and germination were conducted using GERMINATOR software [37], a curve-fitting program designed for the analysis of germination data.

Seed vigor test using accelerated aging testing with saturated sodium chloride solution

Saturated salt accelerated aging (SSAA) testing: The SSAA test was adapted and modified from Yagushi, et al. [31]. The seed samples were set on a single layer of aluminum screen and placed in a sealed glass jar above 70 mL of saturated sodium chloride solution; thus, the seeds were not in contact with the solution. The test was performed at 42°C for 72 hours. The germination test was carried out after the SSAA test using four 100-seed replications for each sample within 1 hour after removal from the aging chamber. The testing conditions were those outlined in the germination test section above.

Development of an automatic method for rice seed vigor classification via radicle emergence tests using image-processing, curve-fitting and clustering methods

Image acquisition

Data were acquired using a laboratory-scale imaging system described by Joosen *et al.* [32]. Briefly, a digital single-lens reflex camera (Nikon D5200 with Nikkor AF-S 60 mm f/2.8 G Micro ED; Nikon, http://www.nikon.com) was fixed to a repro stand. Two vertically placed fluorescent tl-tubes (150 cm), 1.5 m left and right from the camera, were used as an indirect light source, and great care was taken to prevent any reflection. The camera was set to full manual control (ISO 500, f/16, 1/15 sec, autofocus). A position mask was used to ensure that the trays were placed at the correct position under the camera (see Supplementary material 4).

Image analysis

There were two important steps in the image-processing procedure: seed segmentation and counting of the number of germinated seeds (Figs. 8 and 9). [illegible]ation of the rice seeds from the blue screen background. The counting step determined the number of

germinated seeds in the input image (Figs. 9 and 10).

Since the color of the background and indica rice seeds were blue and yellow tones, respectively, as shown in Fig. 9, the properties of a YCbCr color model were utilized, where Cb represented the difference between the blue portion and luminance value (Y), while Cr represented the difference between the red portion and luminance value. In the seed segmentation step, an input RGB image was transformed to the YCbCr color model according to the following equations.

$$\begin{bmatrix} Y \\ Cb \\ Cr \end{bmatrix} = \begin{bmatrix} 0.257 & 0.501 & 0.097 \\ -0.147 & -0.291 & 0.438 \\ 0.438 & -0.368 & -0.071 \end{bmatrix} \begin{bmatrix} R \\ G \\ B \end{bmatrix} + \begin{bmatrix} 16 \\ 128 \\ 128 \end{bmatrix}$$

where Y is the luma component and Cb and Cr are the blue-difference and red-difference chroma components. An RGB color is made from three colored lights: red, green, and blue.

The image-processing procedure involved four main steps, as shown in Fig. 8„: 1. group of seed localization, 2. seed segmentation, 3. germinated seed segmentation, and 4. germination detection. An example of an input image is shown in Fig. 9a. The black-white and some blue backgrounds included irrelevant regions. To reduce computation time and complexity, the seed and germinated seed regions should be localized. In step 1, the input image was transformed to the YCbCr color model. The Cr component was binarized using Otsu's multithresholding [42], morphology closing was applied, and the largest region was selected and cropped. The results of this step are shown in Fig. 9. The cropped input image was again transformed to the YCbCr color model. In step 2, to segment only seeds (without radicles), the Cr component and Otsu's multithresholding were applied. In step 3, to segment the seeds with radicles, the Cb component and Otsu's multithresholding were also applied. The example results in steps 2 and 3 are shown in Fig. 9c and 9d, respectively. In step 4, the blobs of germinated seeds were considered individually. The hypothesis was that the number of remaining objects in the subtracted image between blobs in step 3 and step 2 (in the same region of blobs in step 3) would be the number of germinated seeds. In this step, we computed three values: the single seed area, the number of seeds in each blob, and the number of germinated seeds in each blob. The single seed area was computed by dividing the number of white pixels of the seed segmentation image (result from step 2) by the total number of seeds from the input image. Note that 100 seeds were planted in each tray. The number of seeds in each blob was computed by dividing the number of white pixels in each Cr (in the same region of the Cb blob) by the single seed area. Then, the number of germinated seeds in each blob was counted as the number of remaining objects of the subtraction between the Cb blob and the morphological dilation of the Cr (in the same region of the Cb blob). An example result of the subtracted blob image is shown in Fig. 9e. It should be noted that the number of germinated seeds in each blob must be less than or equal to the number of seeds in each blob. Finally, the number of germinated seeds in each blob was summed to be the number of germinated seeds.

Seed radicle emergence curve fitting and clustering

The radicle emergence data and time intervals were transported to the counting tables (Figs. 8 and 10). Final cumulative radicle emergence tables were automatically loaded into the curve-fitting module using the four-parameter Hill function, and radicle emergence parameters were extracted. Seed vigor was classified using K-means and silhouette analysis through multiple parameters of radicle emergence parameters extracted from the curve fitting.

Data analysis

Inferential statistical analysis was based on analysis of variance (ANOVA) with a single-factor (fixed effect model) and was performed on the results at a significance level of $p \leq 0.05$ followed by Tukey's honest significant difference (Tukey's HSD) post hoc tests to identify significant differences among means. Homogeneity of variance (Levene test) and normality of data were tested in accordance with the assumptions for ANOVA. The percentage data from the software were angularly transformed before ANOVA was carried out (transformed by arcsine$\cdot\sqrt{x/100}$); untransformed values are shown in Table 1 to facilitate comparison). The K-means algorithm was used for cluster analysis, while silhouette analysis was used for selecting the number of clusters for K-means clustering. Correlations between the SSAA test and the results using SVRice software were calculated using the R software package [43] and plotted using the ggplot2 procedure.

Abbreviations

ANOVA
analysis of variance; AUC:area under the curve of the radicle emergence fitted curve; KDML105:Khao Dawk Mali 105 rice cultivar; MaxRE:maximum radicle emergence; MRET:mean radicle emergence times; RGB:red green blue color model; SE:structuring element; SSAA:saturated salt accelerated aging; SVRice:a software package for rice seed vigor classification using germination phenotyping via image-processing, curve-fitting and clustering methods; t_{50}:radicle emergence speed; U_{7525}:uniformity of radicle emergence; and YCbCr:a luma signal and two chroma component color model.

Declarations

Authors' contributions

The automatic method for rice seed vigor classification via radicle emergence testing using image-processing, curve-fitting and clustering methods was conceived by DO. The germination test and image acquisition were conducted by WP and DO. PS performed the software evaluation and trait analysis. Data analysis and manuscript writing were performed by DO and PS. The manuscript was improved, and suggestions were made by DO, PS and WP. All authors read and approved the final manuscript. DO and PC contributed equally to this work.

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Availability of data and materials

The datasets during and/or analyzed during the current study are available from the corresponding author upon request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

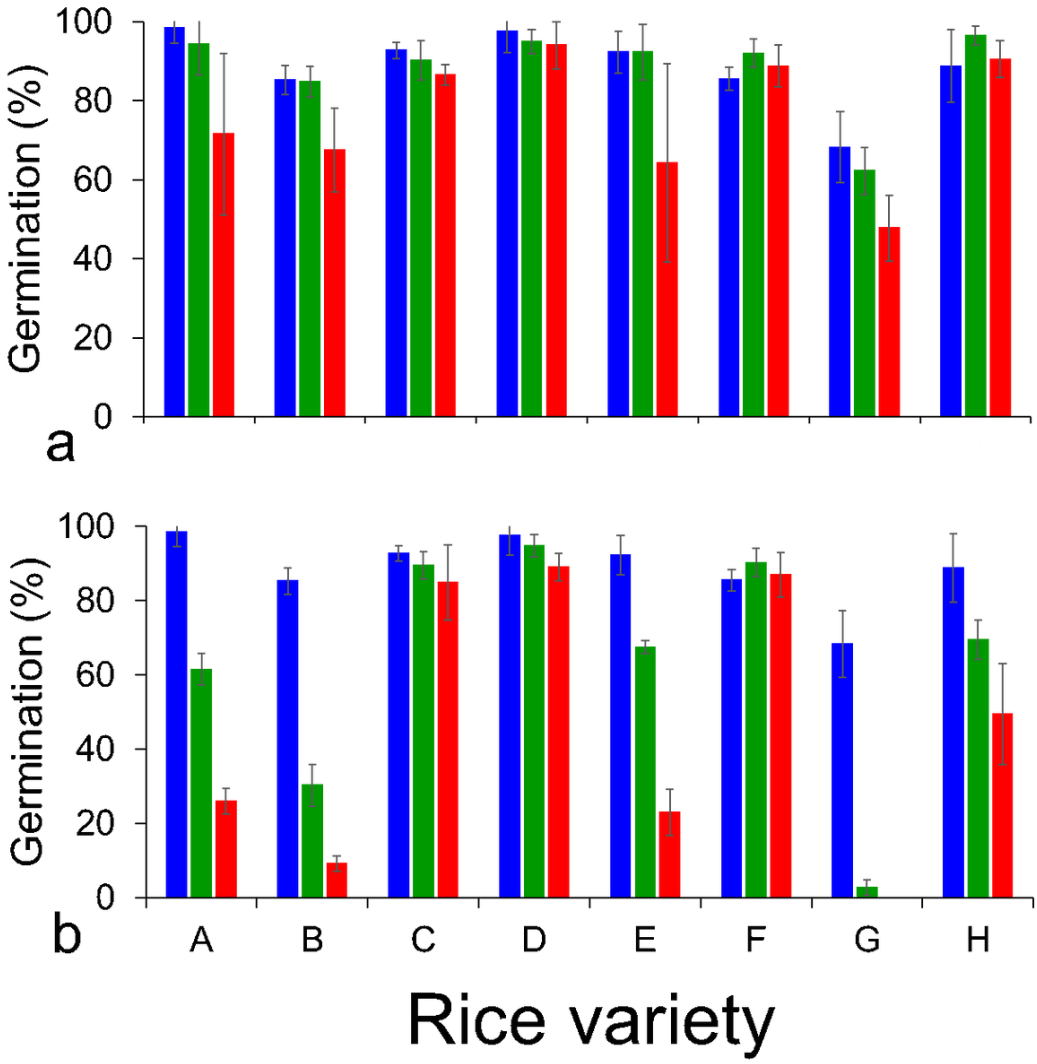

Figure 1

Seed germination of various indica rice varieties after 12 months of storage under different conditions: a controlled and b ambient. The blue, green and red bars represent the arithmetic mean of percentage germination of samples from 0, 6 and 12 months, respectively. The germinator was set to 25°C. Error bars denote confidence intervals (n = 4; p < 0.05); missing error bars indicate ranges smaller than the symbols.

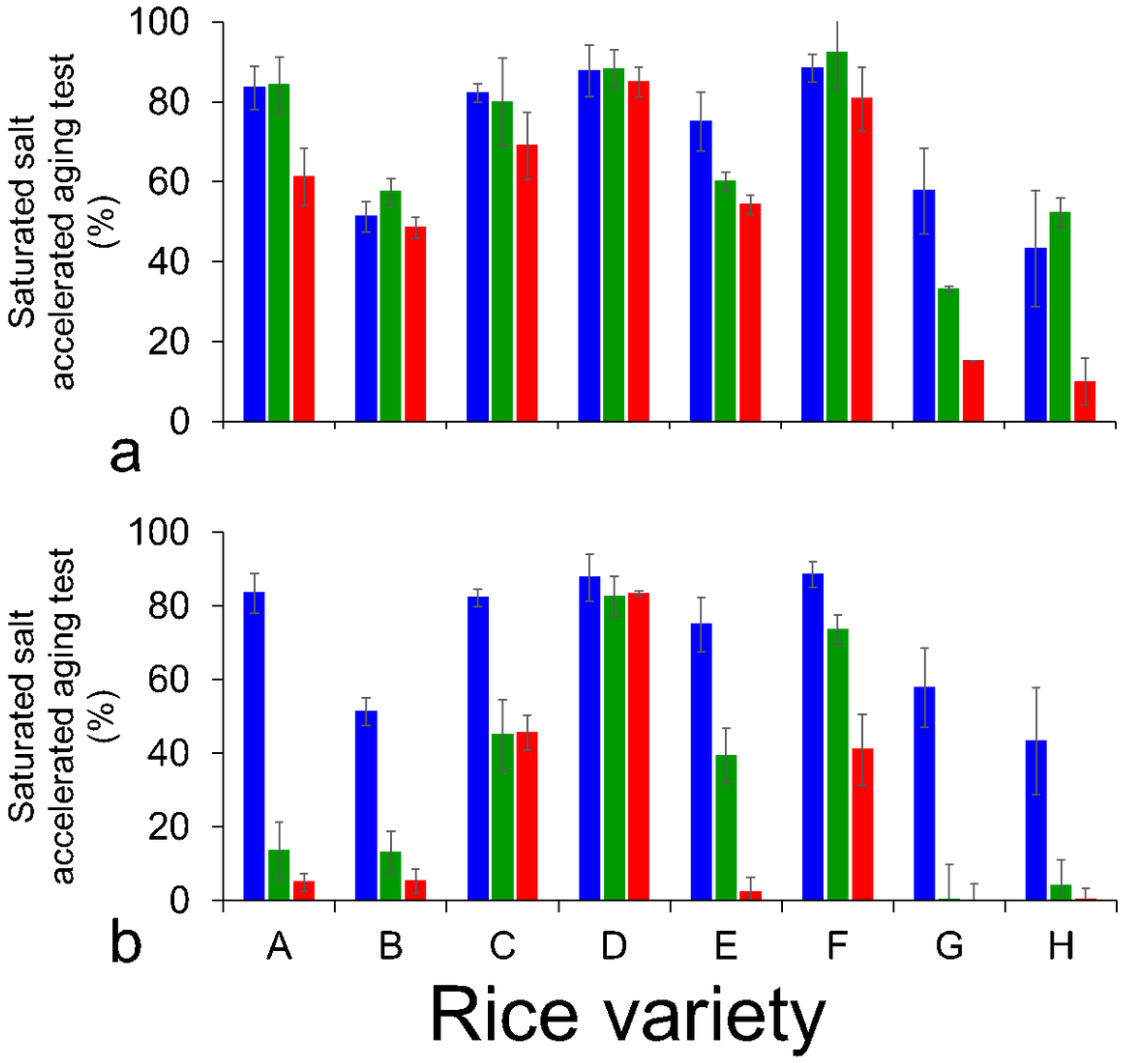

Figure 2

Saturated salt accelerated aging test of indica rice seeds of different varieties after storage under controlled a and ambient b conditions. The blue, green and red bars represent the arithmetic mean of percentage germination of samples from 0, 6 and 12 months, respectively. Error bars denote the confidence intervals (n = 4; p < 0.05); missing error bars indicate ranges smaller than the symbols.

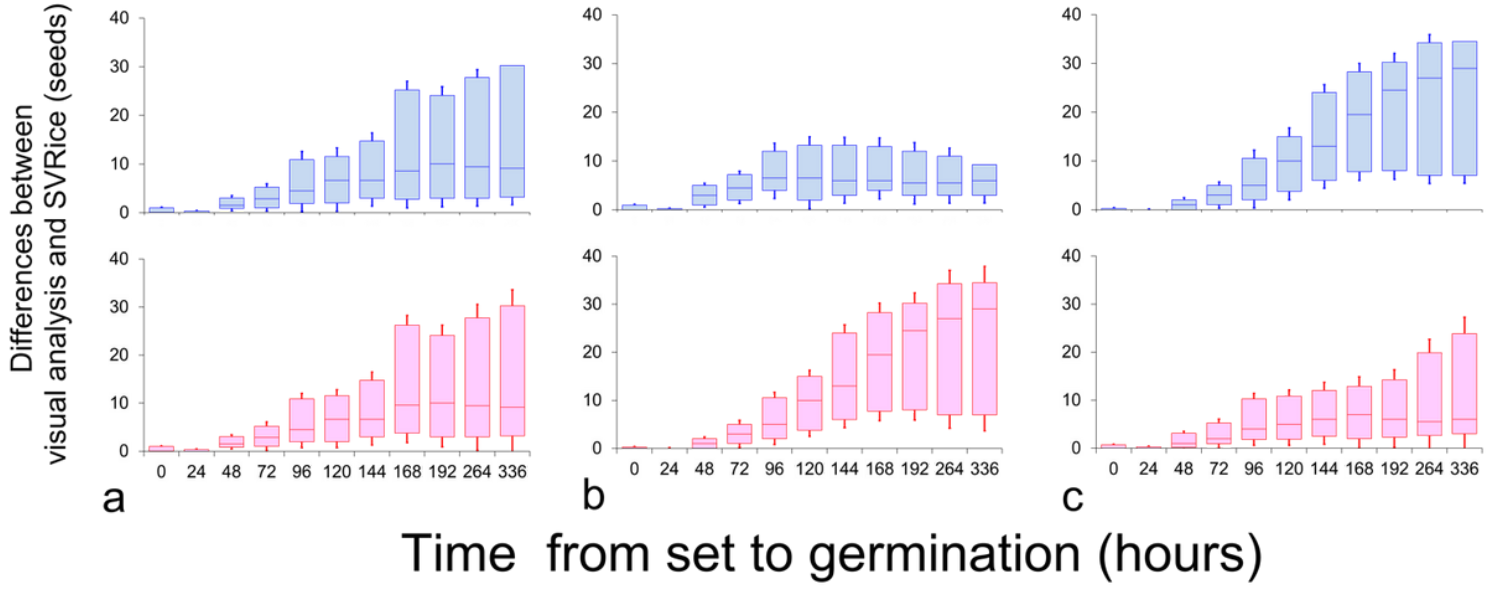

Figure 3

Box plots of differences between detected radicle emergence from visual analysis and SVRice package at different times from set to germination. Blue and red colors represent box plots of differences between visual analysis and the SVRice package under controlled and ambient conditions, respectively. Datasets from 0, 6 and 12 months of storage are represented in a, b and c, respectively. Error bars denote the standard error (n = 32); missing error bars indicate ranges smaller than the symbols.

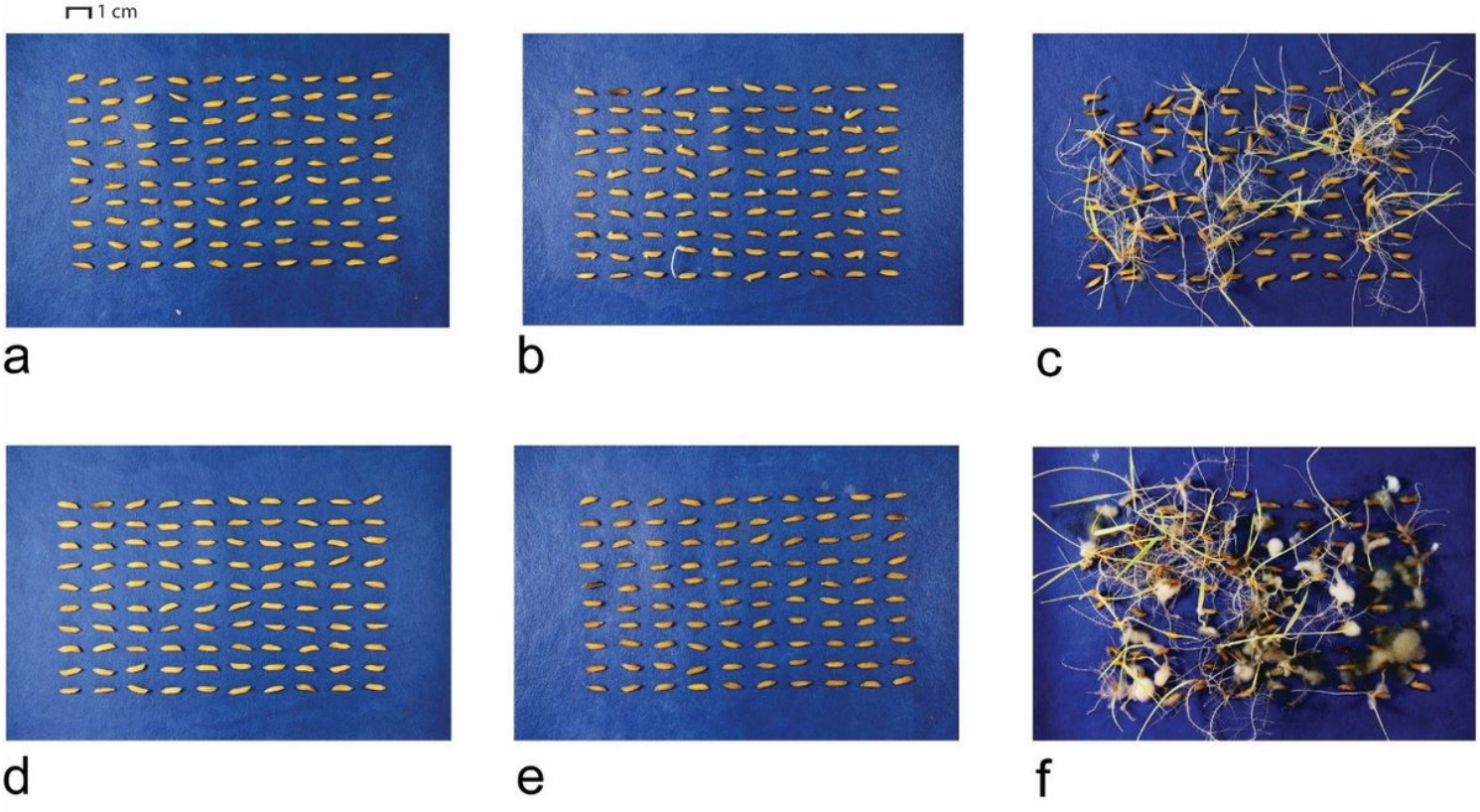

Figure 4

Radicle emergence and germination at 0, 96 and 336 hours after the start of imbibition of indica rice seed code A (a, b and c) and code G (d, e and f) from 12 months of storage under ambient conditions. The germinator was set to 25°C.

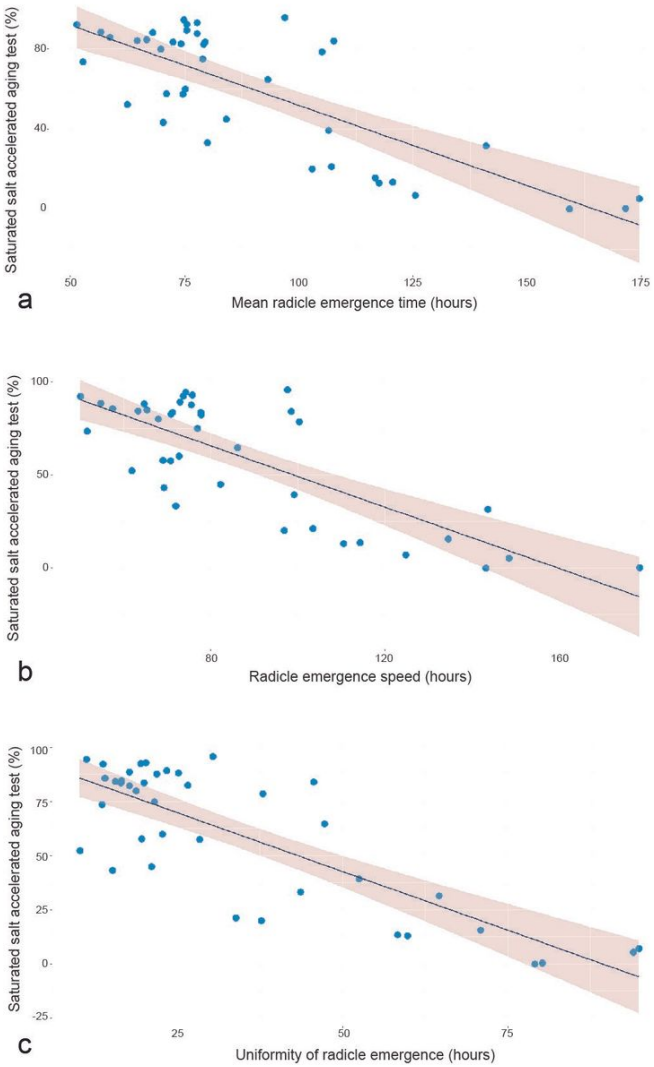

Figure 5

Correlation between various rice seed vigor parameters. a Mean radicle emergence time and saturated salt accelerated aging (SSAA) test, b radicle emergence speed and SSAA test, and c uniformity of radicle emergence and SSAA test. Data derived from SVRice for 40 indica rice seed samples. Pearson's correlation was significant at $p \leq 0.05$. Darker shaded areas indicate confidence intervals of the linear fitted model.

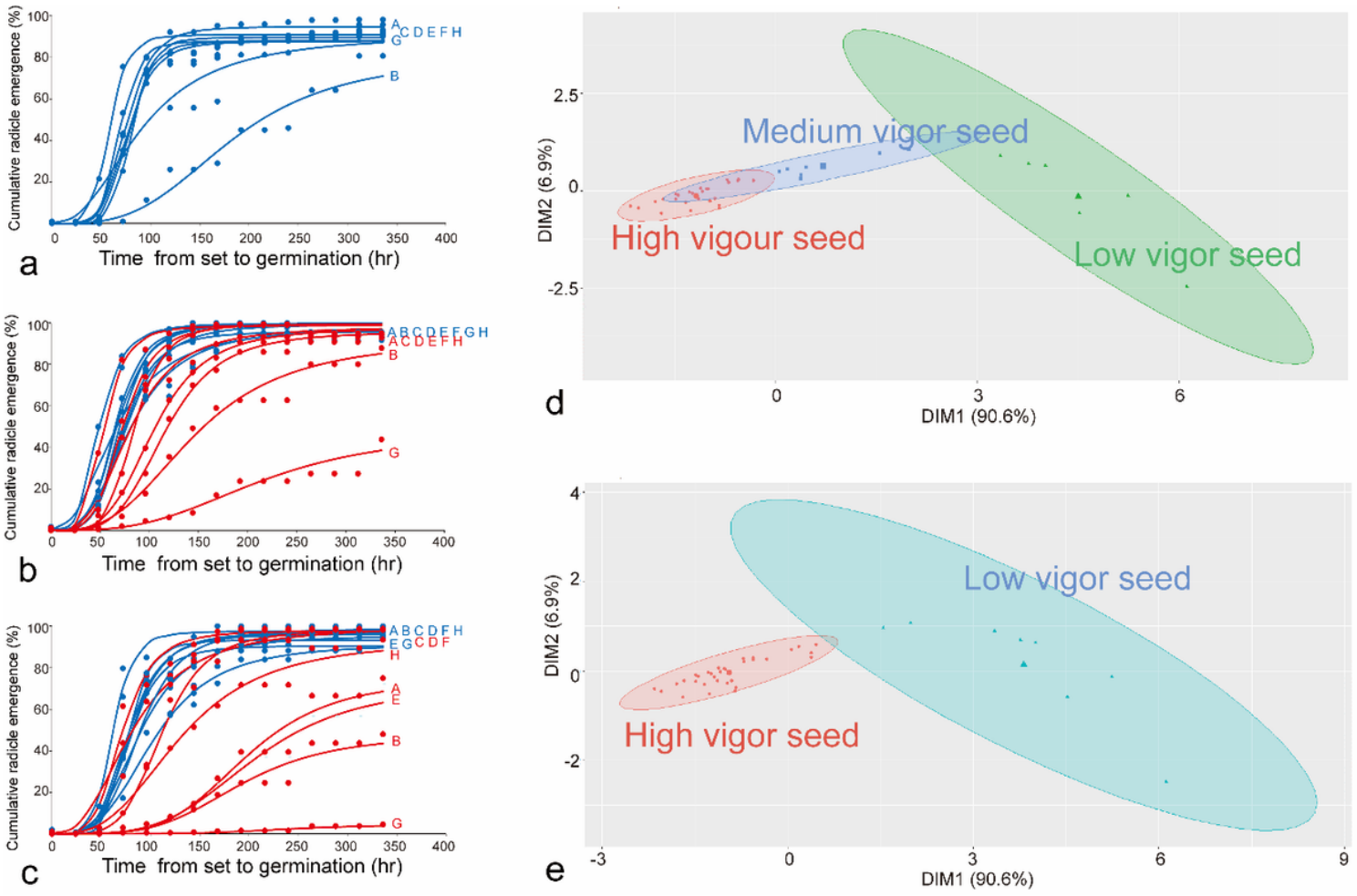

Figure 6

SVRice package results for rapid seed vigor classification of indica rice seeds under different storage conditions and storage times. Blue and red represent the cumulative radicle emergence curves of rice seed samples stored under controlled and ambient conditions, respectively. The germinator was set to 25°C. Datasets from 0, 6 and 12 months of storage are represented in a, b and c, respectively. The K-means algorithm was used for cluster analysis of indica rice seeds using maximum radicle emergence, mean radicle emergence times, radicle emergence speed, uniformity of radicle emergence and area under the curve of the radicle emergence fitted curve for every rice variety, storage time and storage condition. The number of clusters is 3 (d) and 2 (e).

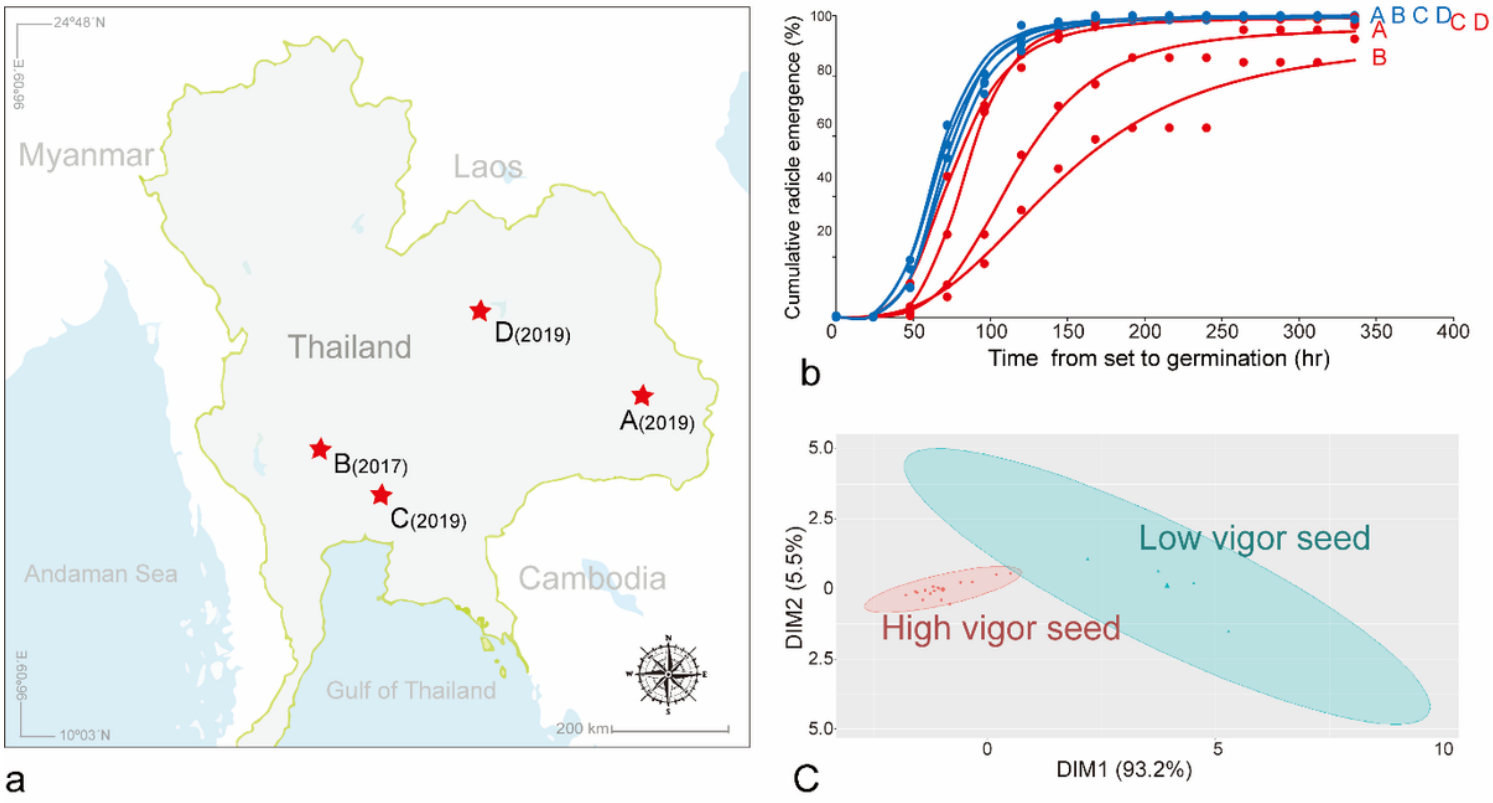

Figure 7

Radicle emergence of KDML105 rice seeds in different production areas and years. a Radicle emergence of KDML105 rice seeds in different production areas and years: Ubon Ratchathani (code A), Suphan Buri (code B), Pathum Thani (code C) and Khon Kaen (code D). b Cumulative radicle emergence curves of KDML105 rice seeds after 12 months of storage under ambient conditions. The germinator was set to 25°C. Blue and red curves represent the arithmetic mean of radicle emergence from the SVRice package after storage under controlled and ambient conditions, respectively. c The K-means algorithm was used for cluster analysis of KDML105 rice seeds in different production areas and years. The best number of clusters was 2.

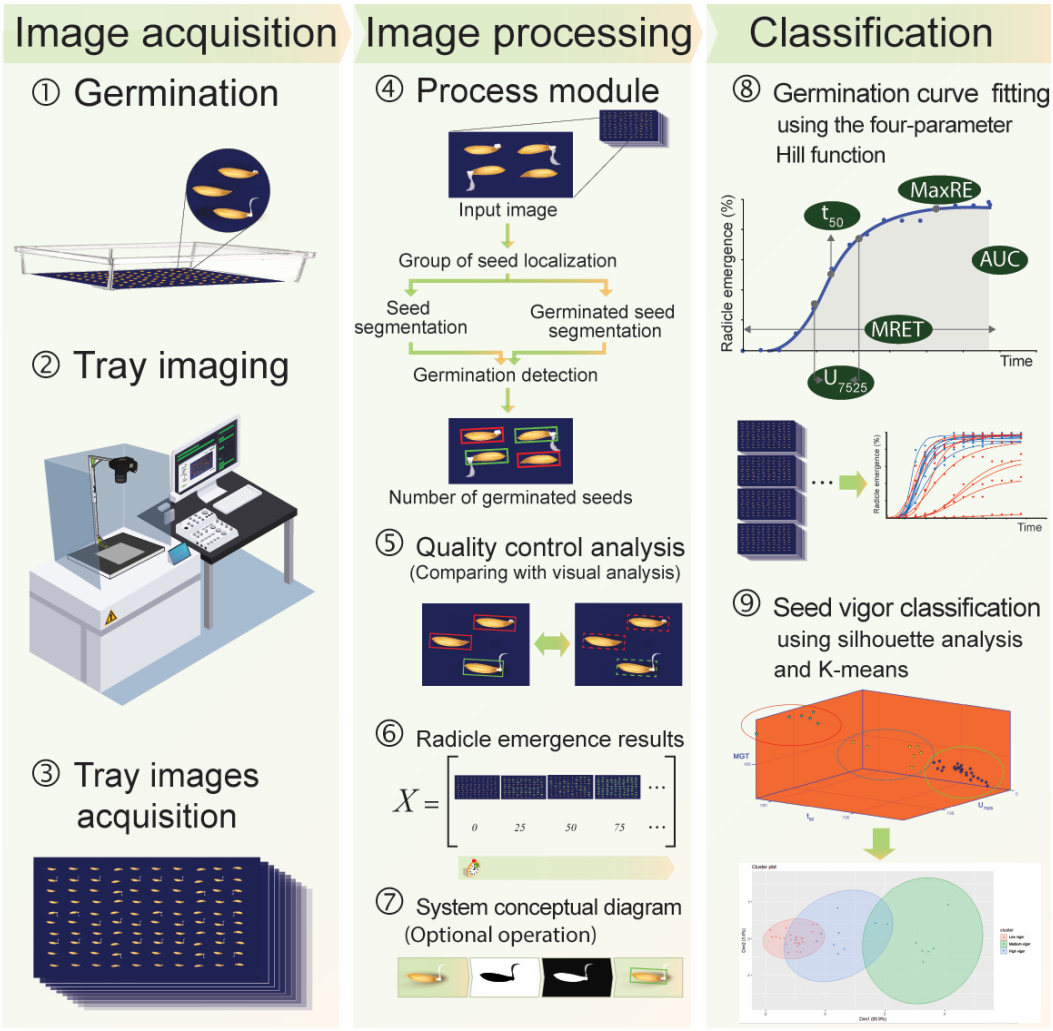

Figure 8

Pipeline for SVRice algorithm development. Representation of the workflow to adapt an algorithm for seed radicle emergence quantification. During the image acquisition phase, germination was stimulated using the top-of-paper technique [1] with four replicates each with 100 seeds on the top of two layers of steel blue seed germination blotter paper, which were placed in a closed polystyrene transparent box and placed in a cabinet germinator (1). Each box was placed under a laboratory-scale imaging system with a DSLR camera for tray imaging (2), and the image was uploaded to the onsite storage progressively during the experiment (3). The example images were transferred to part (4), which consisted of grouping seed localization, seed segmentation, germinated seed segmentation and blob processing in the germination detection step. Seed radicle emergence results were compared with the results of visual assessment for quality control (5). The radicle emergence data and time intervals were transported to the counting tables (6), and users could trace the processing step via a graphic user interface (GUI)-based software application (7). The final cumulative radicle emergence tables could automatically be loaded into the curve-fitting module through the four-parameter Hill function and parameter extraction (8). Seed vigor was classified using K-means and silhouette analysis through multiple parameters of radicle emergence parameters extracted from the curve fitting (9).

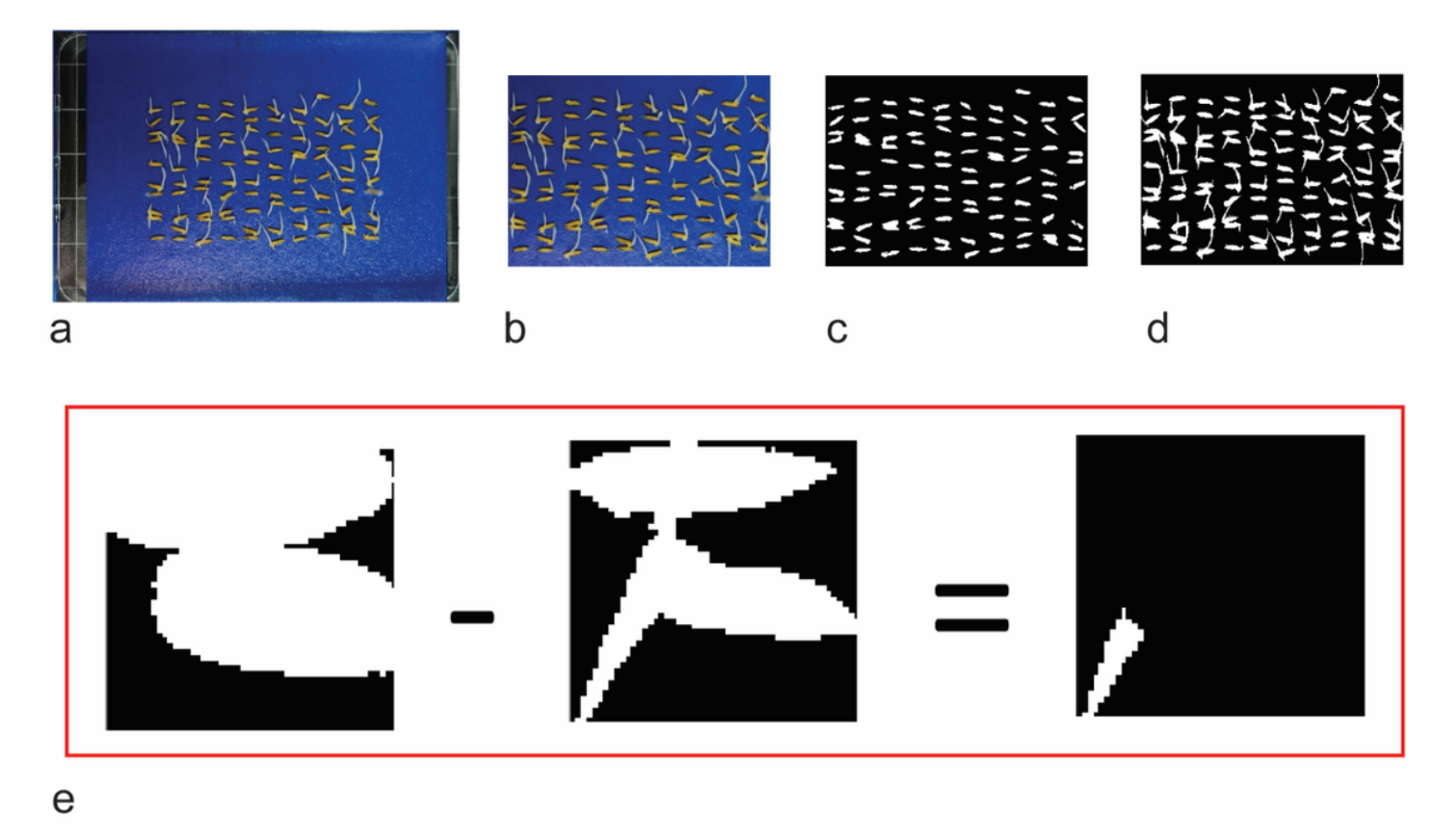

Figure 9

Example images from the image-processing part: a input image, the results from b group of seed localization, c seed segmentation, d germinated seed segmentation and e some blob processing in the germination detection step.

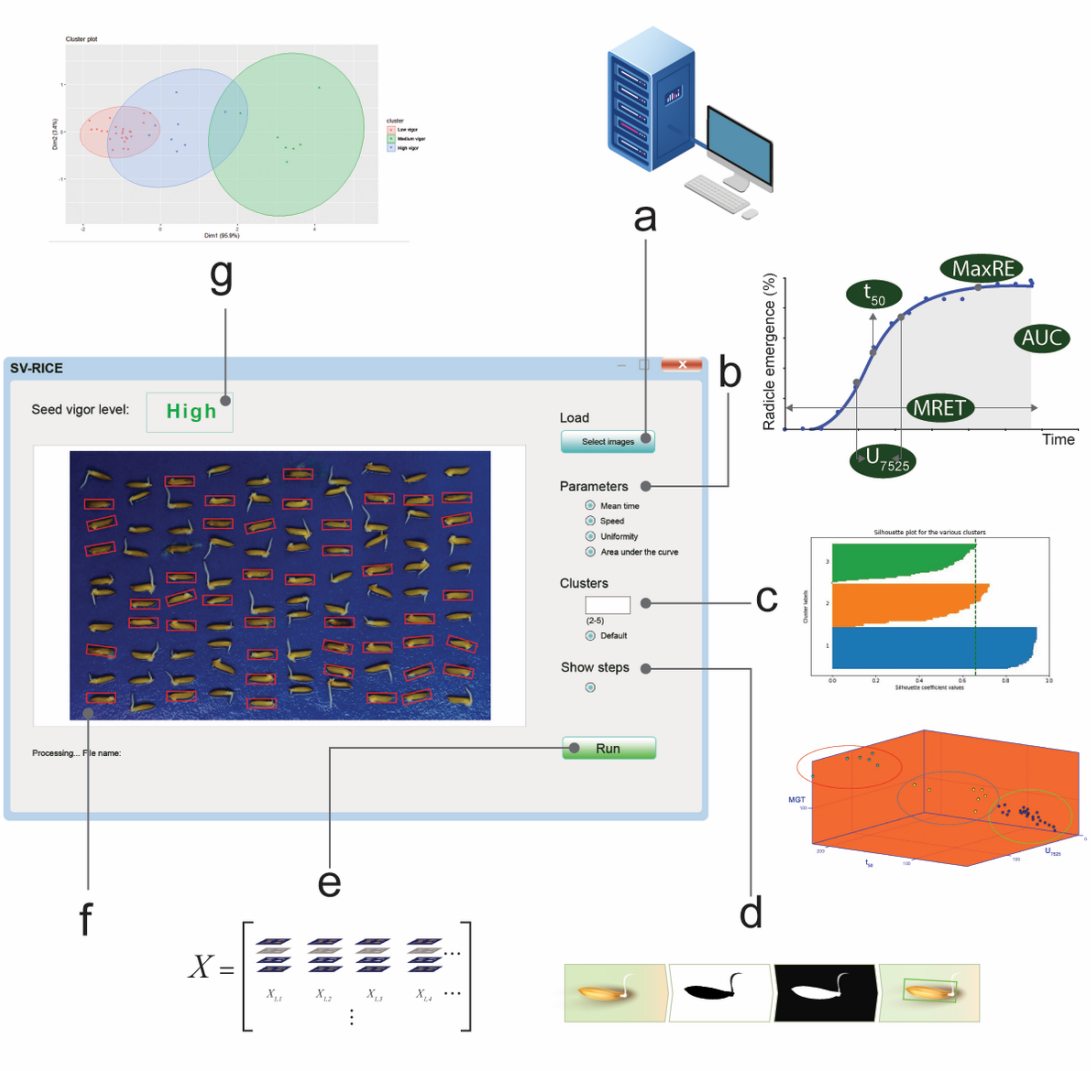

Figure 10

Graphical user interface displays of SVRice software designed for processing multiple radicle emergence image series using image-processing, curve-fitting and clustering methods. a Radicle emergence image uploaded from onsite storage. b The input parameters can be set by users before automated phenotypic analysis and then background pixels in the germination panels (i.e., blue blotter paper) identified via YCbCr color space ranges (where Y stands for luma component and Cb and Cr for the blue-difference and red-difference chroma components, respectively). c After processing, the radicle emergence data and time intervals can be automatically loaded into the curve-fitting module. The number of K-means can be set by users for classifying seed vigor levels or they can choose “default” for optimizing the number of groups using silhouette analysis. d The image-processing step can be displayed to track the progress of the analysis. e After running, the images are processed by grouping seed localization, seed segmentation and germinated seed segmentation. f After processing, the radicle emergence results are shown on the screen. g Rice seed vigor level appears when the analysis is complete.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryMaterialsOnwimoletalResubmission.docx