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

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Research Article

Keywords: Oryza sativa, seed vigor, image analysis, radicle emergence

Posted Date: March 13th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-286963/v1

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Abstract

Background: Rice seed vigor classification is important for seed storage management by seed producers and by farmers planning their cultivation activities. Field emergence is a direct method of seed vigor testing but is laborious, time-consuming and subjective. The saturated salt accelerated aging (SSAA) test is often used as an indirect method for rice seed vigor classification in the laboratory. However, the results from such a method are often imprecise. This paper presents the SV-RICE package, a simple, cost-efficient and flexible procedure that utilizes computer image analysis for high-throughput, automatic rice seed vigor classification. SV-RICE consists of 4 steps: dynamic imaging, image processing, curve fitting and clustering. Seed vigor has been classified based on radicle emergence indices, such as maximum radicle emergence (MRET), mean radicle emergence time (MaxRE), 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 strong negative correlation with the SSAA test. The germination time of 90 hours, at 25°C, was sufficient for effective classification based on SV-RICE, whereas the SSAA test takes approximately 400 hours to complete. The SV-RICE software algorithm was set up to be especially suitable for assessment after 6 months under controlled atmosphere storage (at 15°C and 37%RH in hermetic bag). The study showed that SV-RICE could unambiguously classify 40 Indica rice samples with different varieties, production years, production sites, storage times and storage conditions compared to the SSAA test.

Conclusions: This paper confirmed the accuracy, reproducibility and flexibility of the SV-RICE package for automatic seed vigor classification of *Oryza sativa* seeds; however, it is 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. 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 about one week, depending on the species, and are still highly subjective, for example the accelerated ageing test and the elevated partial pressure of oxygen test [1, 4]. Currently, the radicle emergence test—a fast and reliable method to evaluate seed vigor—has been applied in many species [5–8]. Major advantages of this method are convenience and precision. There is also a high possibility of developing an automatic or semi-automatic method for seed vigor testing using image analysis, or machine vision, through a radicle emergence test [9–11].

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 [12–17]. 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 [18–22]. Interestingly, there have been no published reports on a method to integrate radicle emergence testing and image analysis. Such a new process would not require a long germination period nor 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 [23]. Indica rice seed takes about 3–5 days for the radicle to emerge, depending on geographical races [24, 25]. 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 SV-RICE that could unambiguously classify many varieties of rice seeds from various production sites and different storage conditions and storage times. The SV-RICE 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. The SV-RICE 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 indices using cluster analysis. The results from SV-RICE 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

The percentage germination of rice seed stored under ambient conditions (on the laboratory bench) was less than under the controlled conditions over time, especially at 12 months of storage (Fig. 1). Storage of the Indica rice seed under the ambient conditions at the higher humidity and temperature reduced germination more than storage under controlled conditions at 15°C and 37% RH in the hermetic GrainPro® bags (Fig. 1a).

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

Seed vigor classification via saturated salt accelerated aging test

The SSAA test was used as a seed vigor test. The SSAA value (%) represented resistance to hot and humid conditions by the seed. The controlled conditions not only maintained germination of rice seed but also could delay a reduction of 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 SV-RICE package

The SV-RICE 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 these were 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 seed after 12 months of storage under controlled atmosphere conditions was classified into 2, 3 and 2 groups using MaxRE, MRET and t_{50}, respectively, whilst seed after storing under ambient conditions for 12 months was classified into 5, 2 and 3 groups, respectively, using the same indices (Table 1). The results varied according to the radicle emergence index used.

Table 1
Radicle emergence indices for various varieties of Indica rice seed after 12 months of storage under different conditions using image processing assay in SV-RICE

Code	Controlled atmosphere storage*					Ambient storage†				
	Maximum radicle emergence (%)	Mean radicle emergence times (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 times (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 (%)	5.701261	9.587863	8.658553	33.41275	5.650477	5.960406	17.63861	15.49259	28.06585	8.549533

* 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 the Tukey's honest significant difference test

Generally, the SV-RICE package provided a systematic analysis of results, but it was slightly different from visual analysis (see Supplementary material 1). Differences between detection of radicle emergence based on visual analysis versus the SV-RICE package were analyzed using a box plot (Fig. 3) that showed there was an increase according to the germination time. Rice seeds were dispersed on the steel blue seed germination blotter paper using a mask to ensure an accurate and reproducible spacing. Therefore, clustering of seeds was prevented as much as possible. However, after 120 hours rice seedlings were touching each other and this caused clustering, resulting in greater differences between the visual analysis and the SV-RICE software. Interestingly, the SV-RICE package was especially optimized with Indica rice seed stored under a controlled atmosphere at 6 months (Fig. 3b). Nevertheless, the image processing in the SV-RICE 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 SV-RICE 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 radicle emergence of RD6 (Fig. 4e) was less than for KDML105 at the same time (Fig. 4b). The seed vigor of glutinous rice (code G) was lower than another variety (code A) because it of higher values for MRET, t_{50} and U_{7525} but lower values for MaxRE and AUC.

The SSAA test was correlated with a single radicle emergence index derived from the SV-RICE 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 a single radicle emergence index derived from the SV-RICE package was likely to reflect the SSAA test. However, the use of multiple radicle emergence indices together from the SV-RICE package, would 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 the radicle emergence indices.

After clustering using the SV-RICE package, all the 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 can be adjusted as needed, although the appropriate number was recommended according to the silhouette method. Figure 6d illustrates a k-mean cluster diagram for the 40 rice seed samples used in the experiment, which differed in genetics, production and storage time and storage condition. At 0 months of storage, old seed and glutinous rice seeds were classified as having low-vigor (Fig. 6a-c). Generally, the vigor of rice seed stored under controlled conditions was higher than under ambient conditions; the results for each sample are detailed in Supplementary material 2.

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

Discussion

Seed vigor is a notable characteristic that allows efficient pre- and post-harvest management by seed scientists. As a field emergence test, the direct seed vigor test is laborious and resource-consuming [1, 7]. In addition, the results are subjective as they are heavily dependent on the expertise of the tester. Therefore, seed scientists have been trying to find a convenient, economical and standardized method for seed vigor testing [26]. Unfortunately, to date, there has been no report of an universally acceptable single indirect test for assessing seed vigor [6]. 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 the 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 determine seed vigor accurately, including the stress test, electrical conductivity test and seed growth rate test [2, 27, 28]. Other methods, such as fast ethanol assay, do not consider seedling performance but rely on expensive sensors and have not been reported to be successful with rice seed [29, 30].

Recently, the radicle emergence test has been recognized as being effective and fast for seed vigor testing as it is supported by the metabolic repair hypothesis [6]. The 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 [8, 24, 31]. Interestingly, Joosen, et al. [32] offered the 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 SV-RICE 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 analysis results were questionable due to entanglements in the classification. In contrast, the results from SV-RICE could be clearly separated using the five radicle emergence indices (MaxRE, MRET, t_{50}, U_{7525} and AUC) as shown in Fig. 6e. SV-RICE 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) [33].

The germination time of 90 hours, at 25°C was sufficient for effective classification based on SV-RICE, whereas the SSAA test takes approximately 400 hours to complete. These results were consistent with Onwimol, et al. [25], 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 seed was very different for rice seed samples covering the popular rice varieties in Thailand (Fig. 4). The SV-RICE 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 (SE) for morphological dilation in the counting step.

For calibration, 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 the wrong number of seeds in the counting step. The counted seed number might be greater than the number of seeds.

The developed a software is suitable for high-throughput seed vigor classification. The SV-RICE 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. The SV-RICE software can be applied to detect seed health and seed damage from insects during storage in a warehouse, if multispectral imaging is utilized [34]. The SV-RICE 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 of 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 SV-RICE 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 used 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 Indica rice seed samples used in this experiment

Code	Variety	Phenotype description	Harvest time*	Seed producer	Production area location
A	KDML105	Non-glutinous, photoperiodic, low-amylose*, fragrant	2019	Government agency	15° 19' 23.1424" N, 104° 42' 59.8156" E
B	KDML105	Non-glutinous, photoperiodic, low-amylose, fragrant	2017	Private corporation	14° 38' 56.2963" N, 100° 0' 1.5466" E
C	KDML105	Non-glutinous, photoperiodic, low-amylose, fragrant	2019	Government agency	14° 1' 0.7532" N, 100° 43' 37.4297" E
D	KDML105	Non-glutinous, photoperiodic, low-amylose, fragrant	2019	Government agency	14° 32' 5.5464"N, 102° 7' 3.7333"E
E	RD31	Non-glutinous, day-neutral plant, high-amylose†	2019	Government agency	14° 32' 5.5464"N, 102° 7' 3.7333"E
F	PTT1	Non-glutinous, 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	Non-glutinous, 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) that was conducted at 15°C and 37%RH, whilst 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 (ISTA, 2018) with four replicates each of 100 seeds. Seeds were germinated on the top of two layers of steel blue seed germination blotter paper (Anchor®, Minnesota) which are placed in polystyrene transparent box (9.2 × 28 × 5.7 cm^3)

and took closed boxes, then 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 (2018).
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 the GERMINATOR software [32], 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. [28]. 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, so the seeds were not in contact with the solution. The test was performed at 42ºC 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 ageing chamber. The testing conditions were those outlined in section germination tests above.

Seed vigor test using image processing

Image acquisition

Data were acquired using a laboratory-scale imaging system described by Joosen et al. (2010). 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 (ISO500, f/16, 1/15 sec, auto focus). A position mask was used to make sure 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 the number of germinated seeds (Fig. 8). The seed segmentation step involved separation of the rice seeds from the blue screen background. The counting step determined the number of germinated seeds in the input image (Fig. 9).

Since the color of background and Indica rice seed were blue and yellow tones, respectively, as shown in Fig. 4, the properties of a YCbCr color model were utilized, where Cb represents the difference between the blue portion and the luminance value (Y), while Cr represents the difference between the red portion and the luminance value. In the seed segmentation step, an input RGB image was transformed to the YCbCr color model according to 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 for red, green, and blue.

Both the Cb and Cr images were binarized using Otsu’s multiple thresholding in Python® [35]. The binarized Cb and Cr images showed germinated seeds and only the seeds, respectively. Both multiple seeds touching each other and single seeds were called an object in this step. It should be noted that each object from the binarized Cb corresponded to its binarized Cr at the same position and each object was analyzed separately.

According to the characteristic of the objects from the binarized Cb and the binarized Cr images, we assumed that the difference between these objects should be the roots of germinated seeds.
In the step counting the number of germinated seeds, we computed three values: the single seed area, the number of seed in each object and the number of germinated seeds. The single seed area was computed by dividing the number of white pixels of the binarized Cr by the total number of seeds from the input image. The number of seeds in each object was computed by dividing the number of white pixels in each Cr object by the single seed area. Then, the number of germinated seeds was counted as the number of remaining objects as the difference between the Cb object and the morphological dilation of the Cr object. It should be noted that the number of germinated seed must be less than or equal to the number of seed in each object.

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 was angularly transformed before the 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 the SV-RICE software were calculated using the R software package [36] 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; SV-RICE:a software package for rice seed vigour classification using germination phenotyping via image processing, curve fitting and clustering method; t_{50}:radicle emergence speed; U_{7525}:uniformity of radicle emergence; YCbCr:a luma signal and two chroma components color model.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported by the Kasetsart University Research and Development Institute (KURDI), the Thailand Research Fund (MRG5980180) and partially by the Center of Excellence on Agricultural Biotechnology, Science and Technology Postgraduate Education and Research Development Office, Office of Higher Education Commission, Ministry of Education (AG-BIO/PERDO-CHE).

Acknowledgements

The Rice Department, Ministry of Agriculture and Cooperatives (Thailand) kindly provided the rice seed samples. The staff in the Seed Laboratory, Department of Agronomy, Faculty of Agriculture, Kasetsart University, Bangkok, Thailand provided advice and assistance in the laboratory.

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Figures

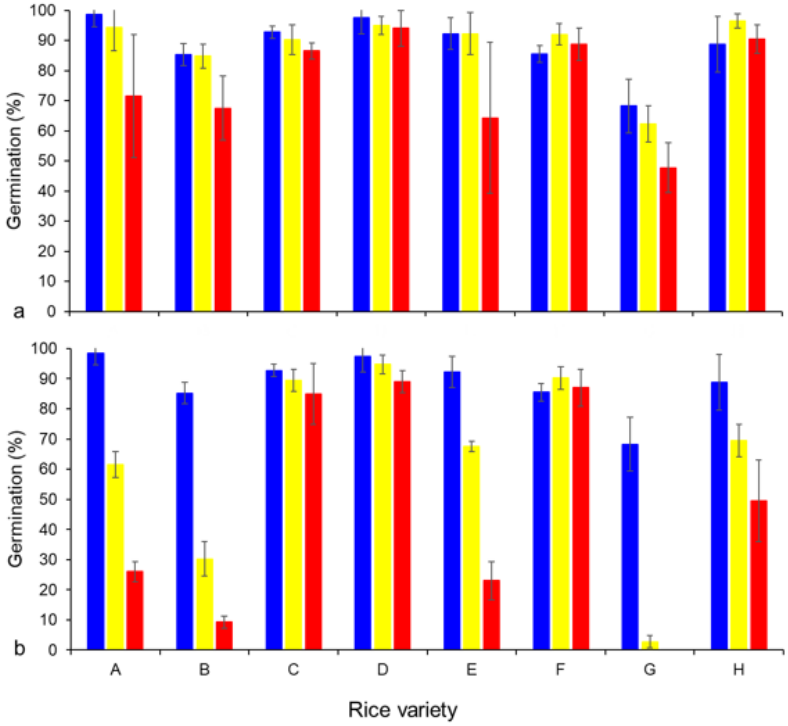

Figure 1

Seed germination of various Indica rice 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.

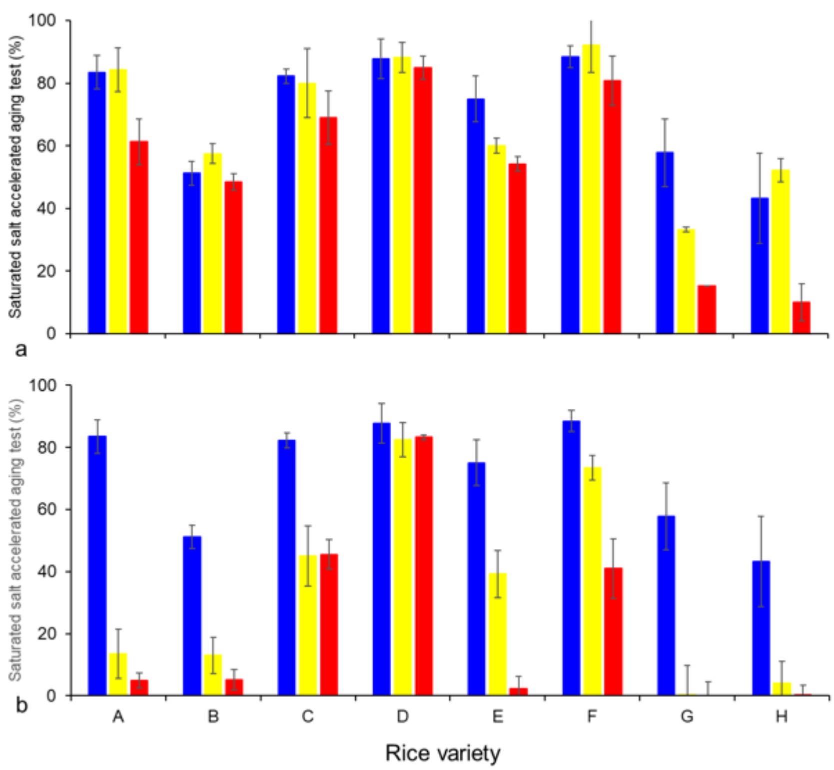

Figure 2

Saturated salt accelerated aging test of Indica rice seed with different varieties after storing in 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.

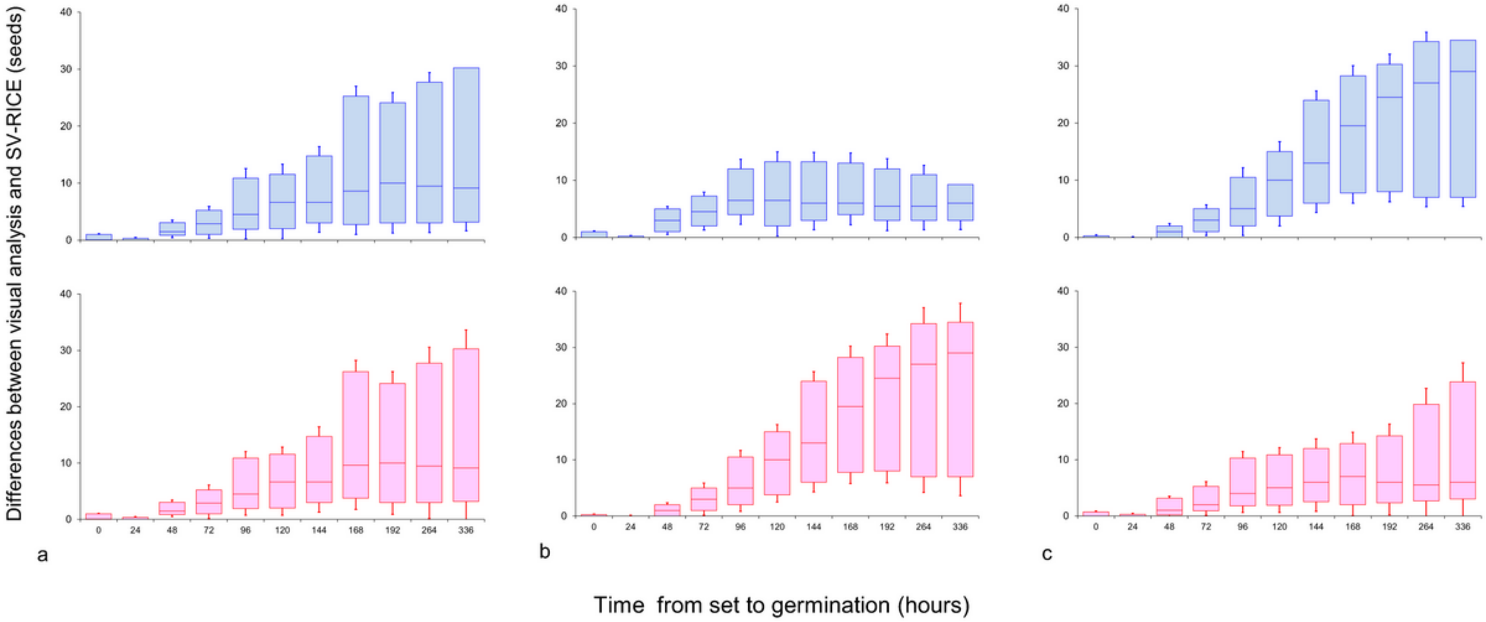

Figure 3

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

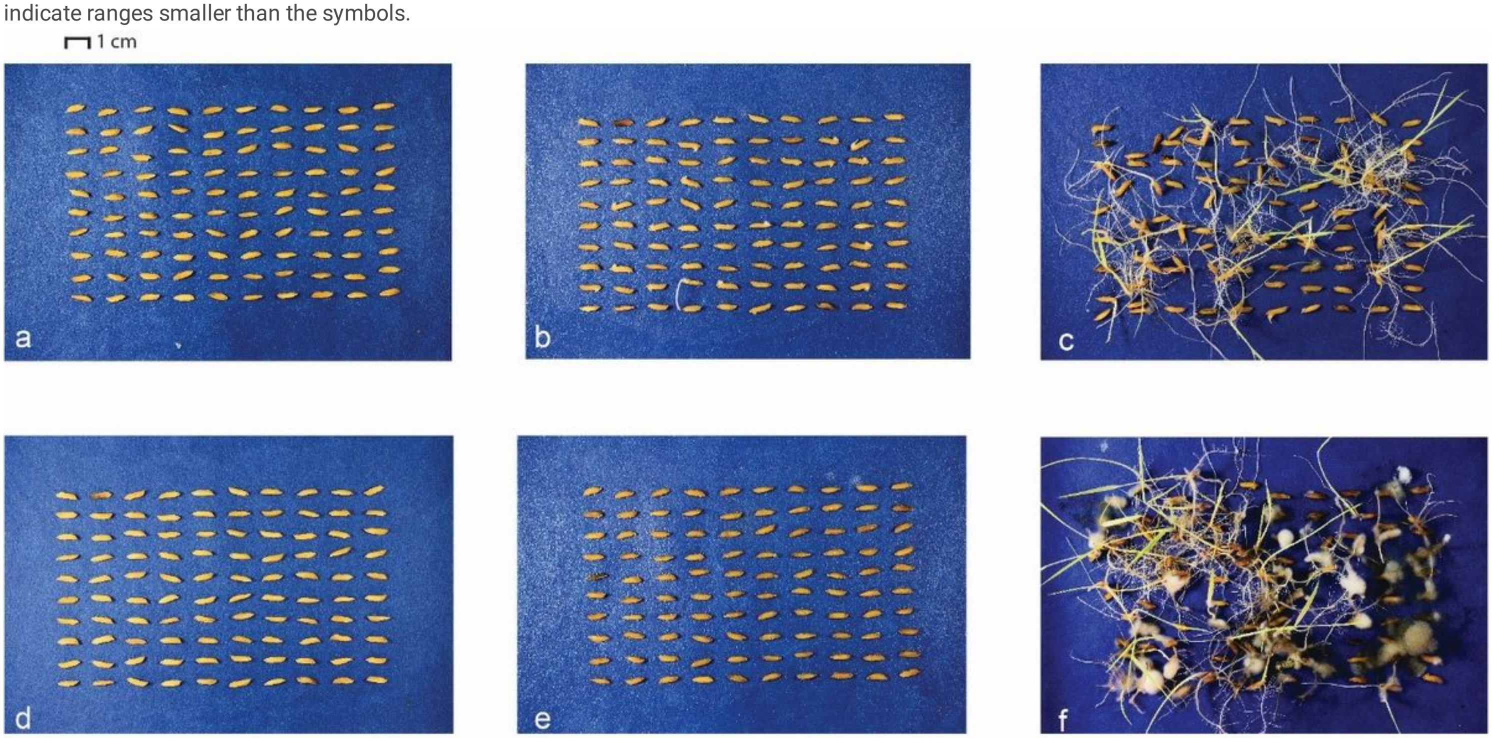

Figure 4

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.

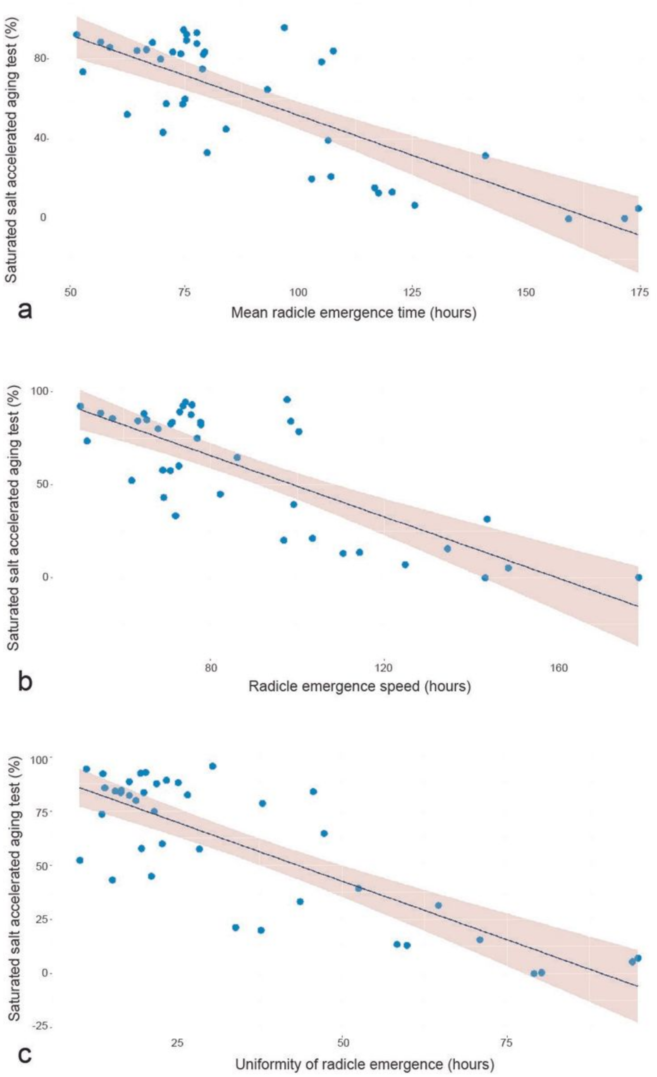

Figure 5

Correlation between various rice seed vigor indices. a Mean radicle emergence time and saturated salt accelerated aging (SSAA) test, b radicle emergence speed and SSAA test, c uniformity of radicle emergence and SSAA test. Data derived from SV-RICE 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 model fitted.

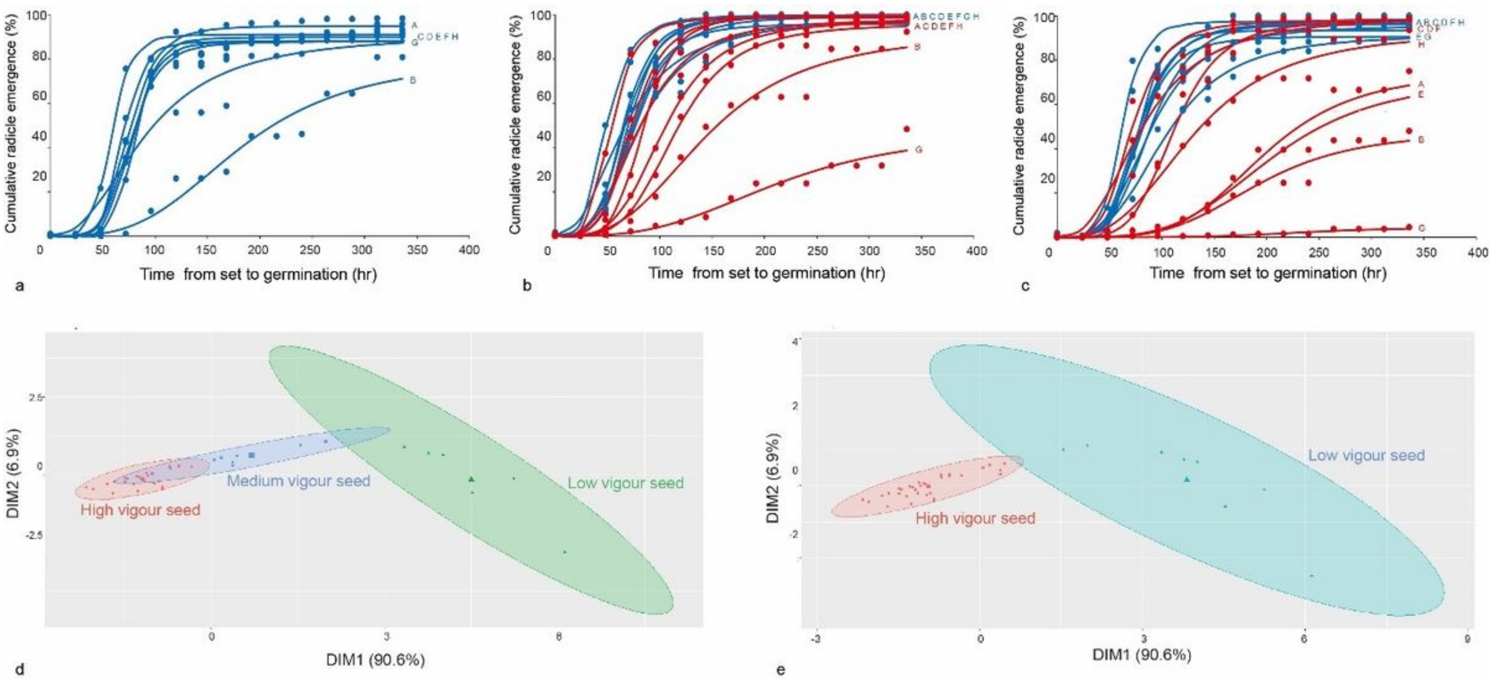

Figure 6

SV-RICE package results for rapid seed vigor classification of Indica rice seed under different storage conditions and storage times. Blue and red represent the cumulative radicle emergence curves of rice seed sample stored under the controlled and ambient conditions, respectively. The germinator was set to 25°C. Dataset from 0, 6 and 12 months of storage represented in a, b and c, respectively. K-means algorithm was used for cluster analysis of Indica rice seed 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).

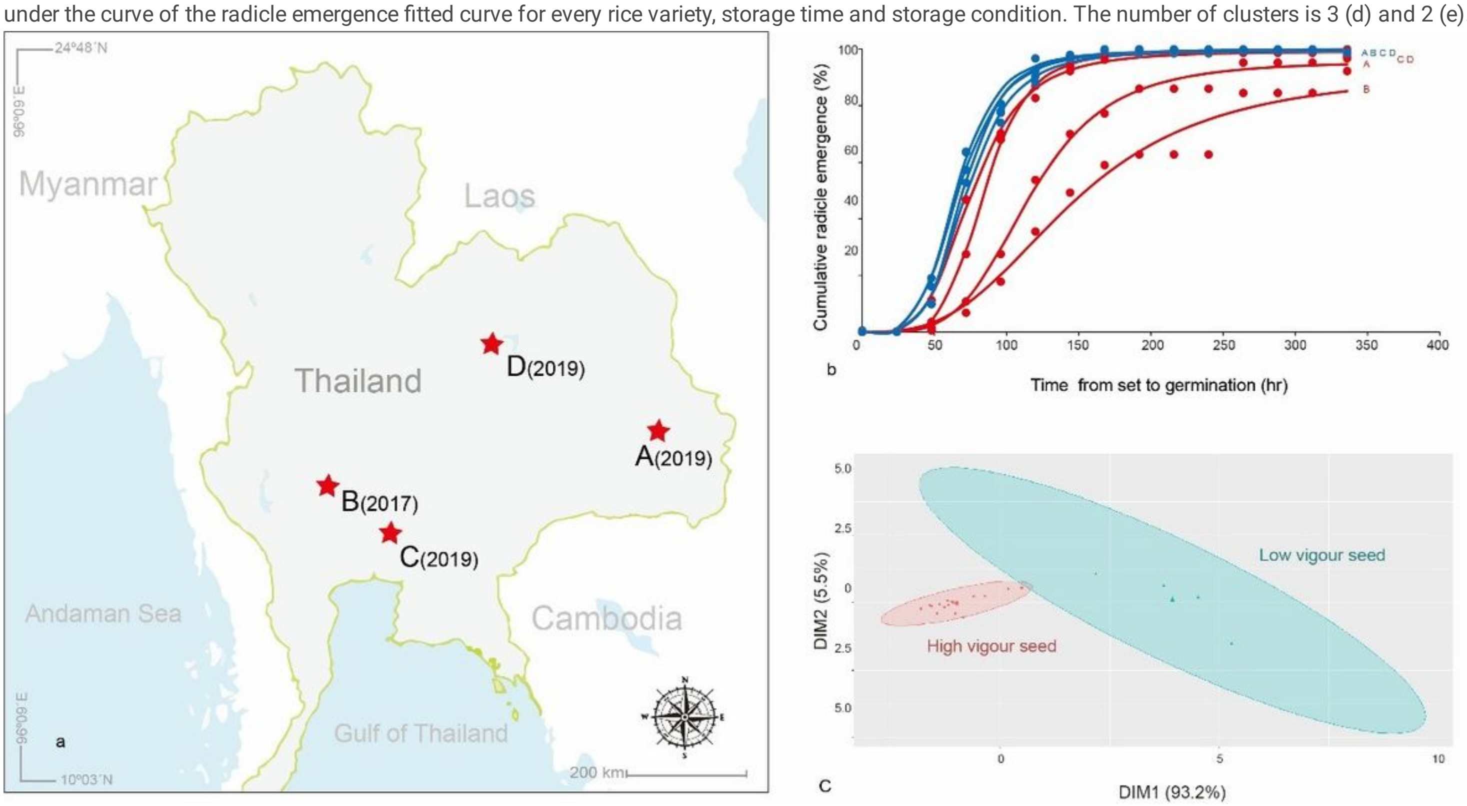

Figure 7

Radicle emergence of KDML105 rice seed in different production areas and years. a Radicle emergence of KDML105 rice seed 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 represented the arithmetic mean of radicle emergence from the SV-RICE package after storage under controlled and ambient, conditions, respectively. c K-means algorithm was used for cluster analysis of KDML105 rice seed in different production areas and years. The best number of clusters was 2. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

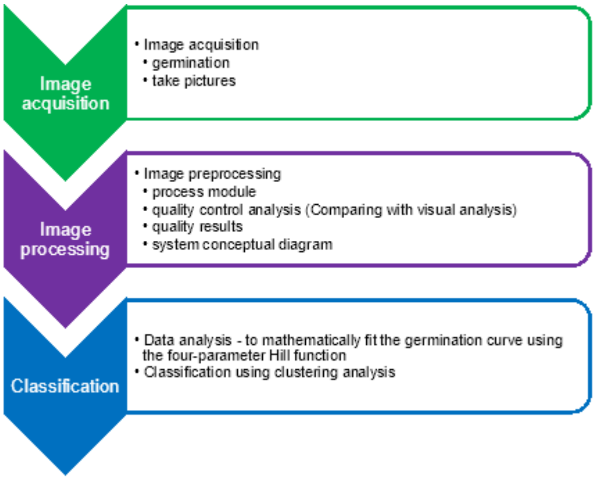

Figure 8

Feature selection and classification steps.

Figure 9

A graphical user interface displays of SV-RICE software.

Supplementary Files

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