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Grain Size Distribution and Head Rice Yield of Some Rice Breeding Lines using Flat-Bed Scanning and Digital Image Analysis

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ABSTRACT

Rice grain quality evaluation is crucial in rice breeding and production of selected varieties. Characterization of rice grain fractions after milling is important; these include brown rice, milled rice, and head rice yield (HRY). The latter is the main determinant for the market price of rice. The present study aims to utilize image analysis as a tool for assessing HRY. Digital image analysis (DIA) was used to quantitatively determine HRY for eight selected rice breeding lines and the results were compared with those obtained by the conventional method of manual inspection (MI). Digital images of the rice grains were taken with a flat-bed scanner (FBS) and analyzed using free-access software (ImageJ). Areas of individual grains were determined, and percent grain area was calculated in terms of the ratio of actual-to-whole grain areas (AWGA). Size grain distribution plots were generated per breeding line, and those grains with values of the AWGA ratio greater than or equal to 75% were classified as head rice. The HRY value was calculated in percent-by-mass basis using the mass of 100 whole rice grains. Values of HRY obtained using DIA showed no significant difference compared to those obtained using the conventional method. This was done according to the paired samples t-test at 95% confidence interval, which showed a high correlation coefficient (R^2 = 0.99). The DIA method showed good repeatability and reproducibility based on different precision parameters indicating its potential as a practical, inexpensive, and quantitative method for determining HRY of rice breeding lines.

Keywords:
Digital photometry, breeding lines, head rice yield, image analysis, imageJ, grain sizing

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Abbreviations: ANOVA - analysis of variance, AWGA - actual-to-whole grain areas, CCD – charge-coupled device, DIA – digital image analysis, FBS – flat-bed scanning/scanner, HRY – head rice yield, MI - manual inspection

INTRODUCTION

The production of rice, which is the staple food in numerous countries especially in Asia, is essential in sustaining billions of people around the world. There has been an increase in the number of studies concerned with the improvement of rice production, not only in quantity but also in terms of grain quality. Rice quality evaluation is of great significance in determining the physicochemical, nutritional, eating, and cooking properties of rice grains. Harvested rice undergoes several processes before reaching the market. These operations include drying, dehulling or removal of the hull to produce brown rice, and milling to remove the bran layer, producing white milled rice (Rehal et al. 2017). During these processes, some grains tend to be broken, reducing the quality of the commodity as a whole, which in turn influences the market price and grade of rice available to consumers (Juliano 2010).

Generally, rice is marketed as long-grain, medium-grain or short-grain, with certain size specifications. Head rice yield (HRY) is the mass fraction of whole milled rice grains (i.e. grains with length ≥75% of the whole grain) that remains after milling (Juliano 2007). For instance, HRY values of 43–51% were recorded for rice varieties developed by the International Rice Research Institute (IRRI), while in the United States, the average HRY value is about 60% (Zhou et al. 2015), and in the Philippines HRY is below the average range milled rice yield of 60–65% (Juliano 2010). The price of head rice can be twice to thrice that of broken rice grains (Rehal et al. 2017); this can be explained by the consumer's perception of broken grains as undesirable (van Dalen 2015). Therefore, determination of the percentage of broken grains is essential in evaluating the quality and price of rice. Indeed, HRY is one of the primary parameters used in evaluating rice milling quality.

Sizing devices have been used to separate broken grains from head rice for HRY determination. Cooper and Siebenmorgen (2007) used a sizing device to separate head rice from broken grains, and HRY was expressed as mass of the head rice over mass of the initial rough rice sample. However, one problem encountered in this method is the difficulty in distinguishing head rice and broken grains especially when the grains are not of the length at which the sizer was designed. In the Philippines, broken grains are also separated using a grain sizer or rice grader, but the operation is also aided by manual separation in order to rectify any inconsistencies in the performance of the machine. Similar to the assessment of other agricultural crops, the evaluation of HRY is traditionally done by manual inspection (MI) or grain counting in the absence of a rice grader or sizing device. Inconsistencies in manual classification and personal bias during manual separation of grains are some of the main problems in manual methods of analysis. Moreover, MI requires highly skilled operators; it is also tedious and time-consuming. Hence, the use of a faster, easier, simple and less subjective method would be advantageous.

Digital image analysis (DIA) has evolved over the years and has become an important and powerful tool in numerous researches. It offers a lot of advantages in comparison to its conventional counterparts; these include different forms of digital cameras, flat-bed scanners and mobile phones. Furthermore, a variety of software has been developed for image processing and analysis, such as ImageJ, MATLAB, Scion and machine vision techniques. Applications of DIA for physical characterization of rice grains have been reported by several researchers (Aghayeghazvini et al. 2009; Aulach and Banga 2012; Antonucci et al. 2014; Hanibah et al. 2014; Birla and Chauhan 2015; Tahir et al. 2015; Kolkure and Shaikh 2017; Pratibha et al. 2017; Heryana et al. 2019; Sethy et al. 2019). Yadav and Jindal (2001) estimated HRY by DIA using ImageTool software. The latter was used to calculate kernel length, perimeter and projected area from the images. The calculated grain parameters were then used to estimate HRY values, which showed good

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correlation with those obtained from a laboratory grader. van Dalen (2005) applied DIA to rice grain characterization using FBS that gave images of a layer of individually separated grains. Compared to digital cameras, FBS cost-effectively provides a large imaging field of view because the small size of sensor array is compensated by the mechanical movement or scanning across the whole viewing area (Göröcs and Ozcan 2014). Furthermore, Yoshioka et al. (2007) has reported that trans-illumination, which happens since the light passes through the layer of grains from underneath, provided a clearer contrast between the parts of the grains compared with illumination from the front.

The ImageJ software used in the present study is an open-source program written in Java programming language for image processing that offers a wide range of plug-ins and macros designed specifically for scientific analysis. Broeke et al. (2015) have compiled studies from numerous disciplines in which ImageJ was employed, such as in the life sciences, astronomy, physics, and engineering. Using the program, colored images can be converted into binary images that contain 8 bits per pixel and can be interpreted as 0 or 1, depending on the intensity value (Burger & Burge 2016). Image to image transformation or morphological image transformation was done by setting the pixels of interest as the foreground pixels while the rest of the pixels are treated as background (Soille 2013). This widely used transformation is called the threshold operator (T). Mathematically, it can be written as Eq. 1, where f is the input image with pixels x. When the values of the pixels lie within the range [ti, tj], a value of 1 is assigned, and the remaining pixels are assigned a 0 value. The threshold operator transforms any 8-bit grey tone image into a binary image. In this representation, the background pixels can be set as black while the foreground pixels are white.

$$[T_{[t_i,t_j]}f(x)] = \begin{cases} 1, & if\ t_i \leq f(x) \leq t_j \\ 0 & otherwise \end{cases} \qquad \text{(Eq. 1)}$$

Applications of DIA in physical characterization of rice grains have been reported by numerous researchers. To date however, there has been no report that deals with the use of DIA for determining HRY for rice breeding lines in comparison with the obtained HRY determined using the conventional manual inspection (MI) method or using rotary rice grader as employed in the Philippine rice breeding program.

In the present study, eight selected local rice breeding lines were evaluated for HRY using flat-bed scanning (FBS) and digital image analysis (DIA) with free-access software (ImageJ). The results showed that the DIA with FBS is reliable and reproducible, but less expensive and more practical compared to the conventional MI method. This indicates great potential of DIA in rice breeding, selection, and grain quality evaluation, and in rice grain size grading for commercial applications.

MATERIALS AND METHODS

Rice samples and experimental set-up

Eight rice breeding lines were selected, coded and obtained from the Philippine Rice Research Institute Los Baños namely GRD 1, 2, 4, 5, 6, 7, 8, and 15. Ten grams of rough rice of these samples were weighed, dehulled, and milled. Head rice grains were separated from milled grains by MI and were weighed to obtain the HRY which ranges from 17 to 41. One hundred whole milled rice grains were counted for each of the samples from the eight breeding lines. The total weight of the grains was determined and recorded as 100-grain weight.

Images of the milled rice grains were obtained using an HP Deskjet Ink (Advantage 2060 all-in-one K110) image scanner. A clear and scratch-free acetate sheet was used as sample holder and a black cardboard, whose size is greater than the acetate sheet, was used to cover the grains in order to exclude stray light.

Digital image acquisition using a flat-bed scanner

The rice grains were spread out as a single layer on a clear acetate sheet and carefully positioned on the scanner glass top, making sure that there is no grain-to-grain contact. A black cardboard was used

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to cover the grains prior to scanning. The procedure is straightforward and almost identical to how a flat-bed scanner is commonly used. For determining different precision parameters, image acquisition was repeated twice in a day; this was also performed on two different days. Image processing and analysis were done with all experimental parameters held constant aside from the time and date of analysis. The measurement of grain areas was done using ImageJ (Version 2x), a public domain Java image processing program.

Determination of HRY by digital image analysis

The digital image was loaded into the program by clicking *File* then *Open* or by dragging the image on the program interface. For measurement of grain areas, the digital images were first converted into grayscale images. The *Image* icon was clicked, followed by *Type* and then the *8-bit* option was chosen. Then, raw grayscale images were converted into binary images by clicking *Process* and then *Binary*, followed by *Make Binary*. The resulting images now contained white rice grains (whole and broken) on a black background. In order to remove dust and other particulate materials that could interfere with the analysis, despeckling was done by clicking *Process > noise > despeckle*. Measurement of the area of each grain was performed by using the *Analyze* option, followed by *Analyze particles*. A dialog box containing several options would appear and the options *summarize, display results, exclude edges, include holes* and *show outlines* were selected. All obtained image data were exported as Microsoft Excel file for data processing.

The extent of grain breakage was expressed in terms of percent grain size and was calculated by dividing the actual grain area by the maximum area of the whole grain (Eq.2).

$$\%\,grain\ size = \frac{area\ of\ the\ grain}{maximum\ area\ of\ a\ whole\ grain} * 100 \qquad \text{(Eq. 2)}$$

The assumption was that the maximum grain area is that of the whole grain. A distribution plot was made using Microsoft Excel 2007 in order to determine the number of grains whose surface areas were within a specified range of values. It was done by creating a “bins” column where the interval of the percentage values was placed. For this study, an increment of five percent was used starting from zero and the frequency of the grains was calculated using the stored frequency formula on the program. The number of head rice grains (i.e. with grain length ≥75% of the whole) was determined from the frequency distribution tables.

HRY determination using ImageJ2x and Microsoft Excel 2007 gave the number of head rice grains as well as the total number of grains in the samples. These values were converted to mass using the 100-grain weight (Eq.3).

$$mass\ of\ head\ rice = \frac{100\ grain\ weight}{100\ grains} * number\ of\ head\ rice \qquad \text{(Eq. 3)}$$

Statistical analysis

Descriptive statistics such as mean, standard deviation, and range were calculated from the obtained data. Paired sample t-test at 95% confidence interval was performed to compare the %HRY values acquired from the conventional manual inspection method and digital image analysis. Precision parameters such as reproducibility and repeatability as well as necessary mean comparisons were also analyzed using the IBM SPSS Statistics Version 23 software.

RESULTS AND DISCUSSION

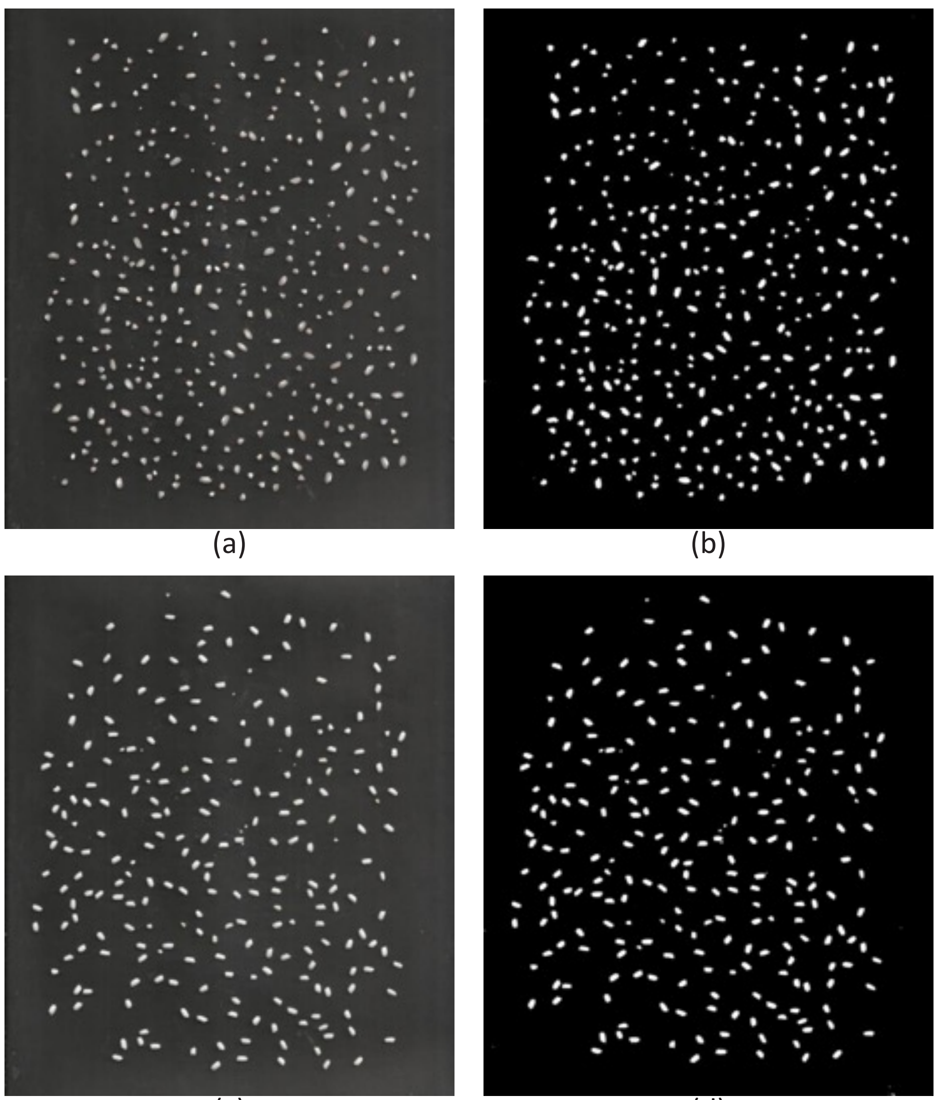
Digital image acquisition

Digital images of rice grain samples were obtained by flat-bed scanning (FBS). In addition to the larger field of view, FBS also enabled capturing images under a more uniform and flicker-free lighting condition, without the appearance of an intensely lit round or rectangular region, which could be the case if an external light bulb or fluorescent lamp was used. Aside from the additional materials used, the process of acquiring rice grain images did not differ from that of getting documents scanned.

Determination of HRY by digital image analysis

Transformation of the scanned digital images into binary images made the analysis easier since only the grains were detected by the program and the background pixels were ignored. Figure 1 shows the difference between the original and the binary images of two breeding lines with the lowest and highest %HRY, i.e. GRD 5 and GRD 15, respectively. ImageJ was able to simultaneously detect, label, count, and measure the area of each grain in all the samples.

Figure 2 shows how each grain was detected and labeled. Since ImageJ measures area and Microsoft Excel counts the number of head rice, another parameter was needed to express the %HRY in milled rice mass basis. For the DIA results to be comparable to the results obtained using MI, the number of head rice was converted to mass using the 100-grain weight. Mean HRY (% by mass basis) of the eight selected rice breeding lines notably showed that DIA was able to detect percent grain areas below 25% (Table 1) which represents the small broken grains according to the Food and Agriculture Organization.



(a) (b) (c) (d)

Figure 1. Original FBS image of sample GRD 5 (a); binary image of sample GRD 5 (b); original FBS image of sample GRD 15 (c); and binary image of sample GRD 15 (d).

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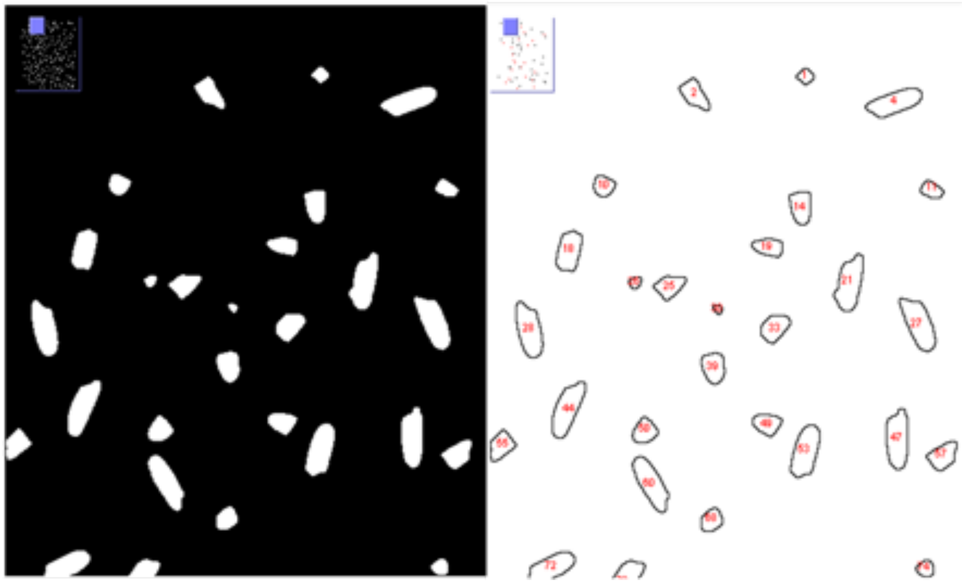

(a) (b)

Figure 2. Enlarged portion of a binary image of a rice sample (a) and image showing how grains are numbered by ImageJ2x (b).

Table 1. Comparison of %HRY by mass mean values obtained using conventional manual inspection (MI) method and digital image analysis (DIA) for eight rice breeding lines, including values of standard deviation (s) and relative standard deviation (RSD) for repeatability (R) and reproducibility (RW) assessment for DIA.

Sample	Head Rice Yield (HRY; % by mass)							
	MI[a,c]	DIA						
		Trial 1[b,d]	Trial 2[b,d]	Trial 3[b,d]	mean[c]	s	RSD_R, %	RSD_{RW}, %
GRD 5	17.0	16.9	16.6	16.6	16.7	0.13	0.92	0.92
GRD 7	23.0	23.6	23.8	23.4	23.6	0.19	0.56	0.56
GRD 1	26.0	26.4	26.3	25.7	26.1	0.38	0.49	1.96
GRD 2	32.0	30.6	30.9	30.9	30.8	0.21	0.82	0.82
GRD 6	36.0	37.5	37.3	37.7	37.5	0.18	0.34	0.33
GRD 8	37.0	37.8	36.4	38.0	37.4	0.89	2.79	0.30
GRD 4	37.5	37.0	38.3	38.8	38.0	0.94	2.40	3.40
GRD 15	41.0	41.2	40.4	40.4	40.6	0.46	1.38	1.38

[a] data obtained from the Rice Chemistry and Quality Laboratory of PhilRice Los Baños
[b] each value expressed as mean of three measurements
[c] not significantly different according to paired samples t-test at 95% confidence interval
[d] not significantly different at 95% confidence interval using ANOVA

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Assessment of DIA as a method for determining HRY

Comparison of mean %HRY using paired sample t-test showed no significant difference between DIA and MI at 95% confidence interval. Precision was expressed as repeatability relative standard deviation (RSDR) for samples analyzed during a short interval of time (Trials 1 and 2) and as within-laboratory reproducibility relative standard deviation (RSDRW) for samples analyzed on different days (Trials 1 and 3). As expected, RSDRW values are generally larger than short-term precision, and all values are within the acceptable range 0.30–3.40%, which is good considering that a portion of the study deals with discreet values (number of rice grains). Furthermore, one-way ANOVA at 95% confidence interval showed no significant difference between the trials performed at different times and days. Linear plots were created to visually show the relationship between the values calculated using DIA with those produced using MI. The results from DIA and MI measurements of HRY were found to have a high linear relationship as shown by the R^2 value of 0.99 (Figure 3). A similar experiment by van Dalen (2005) employed FBS and DIA using a different computer program and obtained repeatable results; a correlation coefficient of 0.98 was obtained when the percentage of broken rice grains was determined using the two methods. Because all of the grains are analyzed at once using a computer program, image analysis using FBS proves to be faster, as well as reducing personal bias associated with the conventional manual inspection. Furthermore, DIA does not require a highly skilled operator to manually examine and separate individual grains; hence, it is accessible to anyone who has a flat-bed scanner and a computer needed for image analysis.

DIA can provide information regarding grain sizing for a rice sample in terms of grain size distribution profile. Figure 4 is a bar graph showing the grain size distribution, in terms of fractions of grain sizes (small broken, large broken and whole grains) of the eight rice breeding lines which were evaluated in the present study in accordance with FAO recommendations (FAO 1992). It should be noted

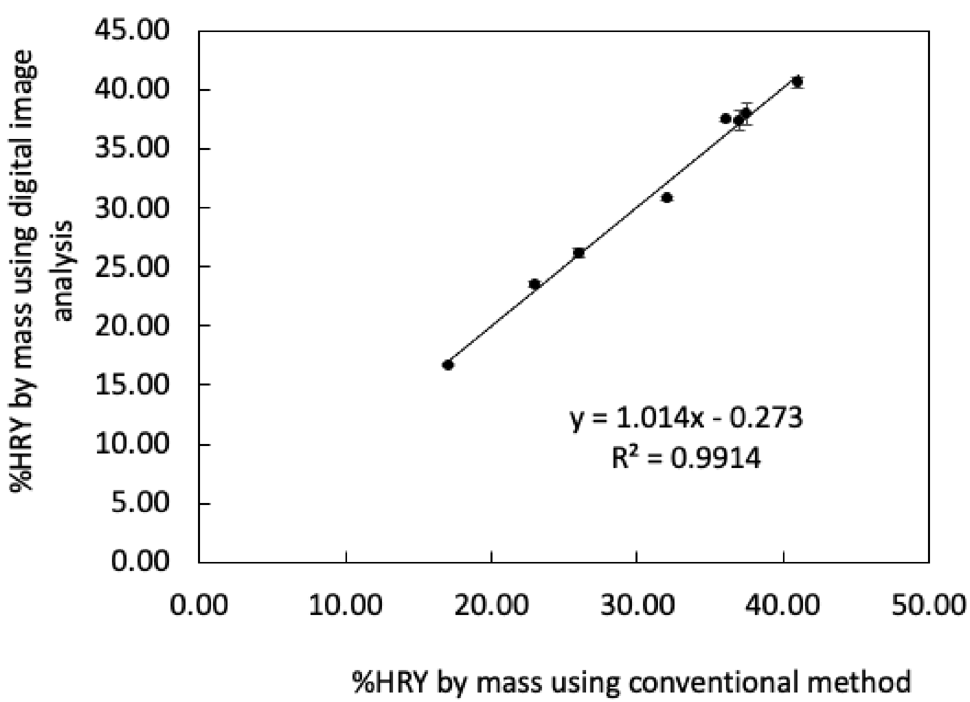

Figure 3. Comparison of %HRY by mass mean values obtained using the conventional manual inspection method and digital image analysis for eight rice breeding lines.

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that for each rice grain, the area calculated from the digital images was the projected surface area of the grain in two dimensions since the grain is perceived by the scanner sensor and then calculated using the ImageJ software. Therefore, the calculated "flat surface" grain area in the image is assumed to be highly correlated with the actual "curved surface" area of the actual grain. However, grain thickness cannot be estimated, thus, 100-grain weight must always be measured prior to HRY determination using DIA. Also, the calculated ratio of actual and whole grain areas was assumed to highly correlate with the mass ratio for the actual grain relative to the whole grain (Eq. 3). The corresponding correlation between the surface area and mass of the rice grains is implicitly proven to be high, based on the HRY obtained in the present study which showed a high positive linear correlation ($R^2 = 0.99$) between the DIA and MI results. Another significant advantage of FBS and DIA in relation to milling potential and grain physical properties evaluation is that digital images of milled rice grains may be stored in a computer and revisited for verification purposes and other software-aided future analyses.

CONCLUSION

Digital images of a layer of milled rice grains of selected breeding lines were scanned with a flat-bed scanner and then stored in a computer. Using ImageJ open-access software the original scans were transformed into binary images and processed; this involved simultaneous area measurement of individual rice grains. Grain-size distribution plots were created and used to calculate the AWGA ratios to aid in the classification of head rice. Head rice yield (HRY) was calculated and expressed as % milled rice by mass relative to earlier measured 100-grain weight. HRY results showed a high positive correlation ($R^2 = 0.99$) between the conventional (MI) and DIA methods. The latter proved to be precise, repeatable and reproducible

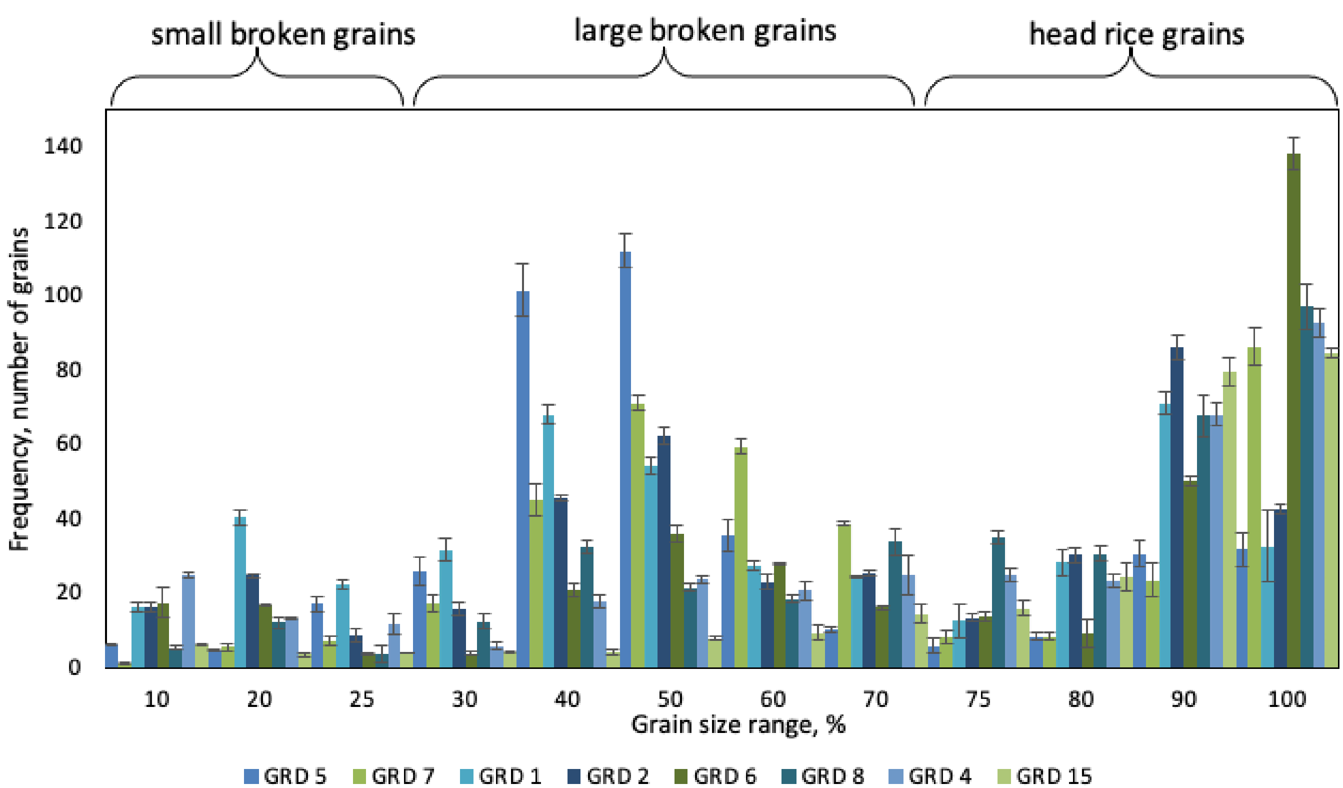

Figure 4. Bar graph showing grain size distribution for the eight rice breeding lines.

which offers a cheaper, more convenient, and faster way to measure HRY of rice breeding lines. DIA can also provide comprehensive information on grain-size distribution of a rice sample, allowing further classification into whole, large broken and small broken grains. DIA shows promise for monitoring grain milling efficiency which dictates the grade and price of milled rice in the market.

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