

DIGITAL IMAGE ANALYSIS USING FLATBED SCANNING SYSTEM FOR PURITY TESTING OF RICE SEED AND CONFIRMATION BY GROW OUT TEST

Analisis Citra Digital dengan Sistem Pemindai Datar untuk Pengujian Kemurnian Benih Padi dan Dikonfirmasi dengan Grow Out Test

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ABSTRACT

The common method used for purity testing of rice seed is human visual observation. This method, however, has a high degree of subjectivity when dealing with different rice varieties which have similar morphology. Digital image analysis with flatbed scanning for purity testing of rice seed was proposed by investigating the morphology of rice seeds and confirmation by grow out test (GOT) in the field. Two extra-long seed varieties were used in this study including a red rice Aek Sibundong and an aromatic rice Sintanur. The identification on 14 parameters of morphological characteristics indicated that only six parameters were correlated, i.e. area, feret, minimum feret, aspect ratio, round, and solidity. The purity of rice seed can be effectively determined using digital image analysis of spikelet color and shape. Based on the discriminant analysis of the digital image the recognition rate of rice seed purity was higher than 99.2% for shape and 93.55% for color. The method, therefore, has a potential to be used as a complement in rice seed purity testing to increase the accuracy of human visual method and it is more sensitive than GOT.

[**Keywords:** Digital image analysis, flatbed scanning, grow out test, seed purity testing]

ABSTRAK

Metode yang biasa digunakan dalam pengujian kemurnian benih padi adalah pengamatan dengan mata telanjang. Namun, metode pengujian tersebut memiliki tingkat subjektivitas tinggi terutama ketika digunakan untuk varietas padi yang berbeda tetapi memiliki morfologi yang sama. Analisis citra digital dengan pemindai datar dapat digunakan untuk menganalisis kemurnian benih berdasarkan morfologinya yang dikonfirmasi dengan grow out test (GOT) di lapangan. Penelitian menggunakan dua varietas padi yang mempunyai bentuk gabah ekstra panjang, yaitu varietas padi beras merah Aek Sibundong dan beras aromatik Sintanur. Identifikasi berdasarkan 14 parameter morfologi menunjukkan hanya 6 parameter yang berkorelasi, yaitu area, feret, minimum feret, aspect ratio, round, dan solidity. Kemurnian benih padi dapat ditentukan berdasarkan warna dan bentuk spikelet yang berasal dari citra digital. Berdasarkan analisis diskriminan dari citra digital, tingkat akurasi pengenalan kemurnian benih padi dengan pemindai

datar mencapai 99,2% untuk bentuk dan 93,55% untuk warna. Dengan demikian, metode ini memiliki potensi untuk digunakan sebagai pelengkap metode konvensional dan lebih sensitif dalam mengidentifikasi kemurnian benih dibandingkan dengan metode GOT.

[**Kata kunci:** Analisis citra digital, grow out test, uji kemurnian benih, pemindai data]

INTRODUCTION

Rice is the staple food for almost half of the global population. Sustainability of rice production is, therefore, important to assure the supply of the food to the growing world population. One of important technologies in world rice production is the high yielding varieties.

The availability of good quality seed of modern varieties is crucial in rice cultivation. The main indicator of quality seeds is a purity. Seed purity is influenced by cultivation techniques, harvesting, transportation and post-harvest processing (Ilyas 2012). In addition to ensure the quality, seed purity is one of the values that should be displayed in the seed certificate label.

Identification and verification of seed purity are commonly based on human vision observation. According to Mulsanti (2011), the technique is less efficient and very subjective among analysts. Advances in computer technology have been applied to replace human visual observations. In addition to the relatively cheap cost, nondestructive and objectivity can be improved.

Currently many computer hardwares are used for supporting image analysis with different purposes. In rice, image process has been used to identify milled rice derived varieties based on their surface colors and texture (Adnan et al. 2015) and to screen salinity tolerance traits using the LemnaTec 3D Scanalyzer system (Hairmansis et al. 2014). However, application

of image-based phenotyping in the development of rice remains limited. The applied image analysis uses camera for assessing the quality of rice seed and counting the number of seeds (Maheshwari 2013). Lurstwut and Pornpanomchai (2017) used a rice seed image which was taken by mobile phone for evaluating seed germination. Nurcahyani and Saptono (2015) used a smartphone for identifying husked rice quality with 96.67% accuracy. Kuo et al. (2016) modified cameras and microscopes to identify rice quality and resulted 89.1% accuracy. Chaugule and Mali (2016) used a special camera to classify rice seeds based on seed angle.

Many image processing tools are now available for different purposes. One of potential image processing tools is flatbed scanner which is available in most laboratories the tool is easy to use, simple, stable in taking pictures and not independent on external light conditions. Image analysis with flatbed scanning system grew rapidly over the last few years. However, the analysis has not been widely applied in seed science and technology. Some researchers reported the use of the method for determining high quality fish fillets (Grassi et al. 2018), distinguishing the healthy and *Fusarium*-infected grains of wheat (Ropelewska and Zapotoczny 2018), measuring pore size distribution of metal foams and porcine trabecular bone (Doktor and Kytir 2010), knowing the difference between air, asphalt and aggregate in asphalt (Tielmann and Hill 2018), and identifying the quality of 16 types of pork and poultry meat (Zapotoczny et al. 2016).

Studies using flatbed scanner on rice seeds include identifying rice seeds of the same variety based on surface shape and texture (Adnan et al. 2015) and measuring the color of Chinese black rice (Kaisaat and Keawdonree 2017). However, confirmation of image analysis results with other methods has not been found. The study aimed to analyze the image using a standard flatbed scanner to identify rice seed purity based on shape, texture and color, and to confirm the results using the grow out test (GOT) in the field.

MATERIALS AND METHODS

Rice Varieties

Two rice varieties, Aek Sibundong, red pericarp; and Sintanur, aromatic rice were used in the study. The differences between the two varieties were important to evaluate the effectiveness of the method through grow out test.

The seed samples with 13% moisture content were collected from nucleus seed (NS) production unit of the Indonesian Center for Rice Research (ICRR) in 2014. The seeds were stored in seed storage facility at 10 °C temperature and 45% humidity. Nucleus seeds

were under the breeders' supervision for purity, true to type and authentic and produced based on ISO quality management system.

Seed Purity Testing Using Image Analysis

A total of 2400 seed samples of each variety (Figure 1) were used for image analysis. Images were captured using flatbed scanner (Canon Pixma MP287). The seeds were spread on the glass plate of the scanner and covered with black fabric sheet before scanning. The captured image was processed and analyzed using Image J software (Schneider et al. 2012) and saved in JPEG format.

Morphological Trait Measurement

Image segmentation was processed using Image J software by changing a true color RGB image to a grey-scale image. The grey-scale image was then converted into a binary image. The binary image was then converted into a black and white image using a binary technique (Figure 2).

Morphological traits of the seeds were determined by measuring the surface area, perimeter, major axis, length of minor axis, angle, BX, BY, circularity, feret, feret x, feret y, feret angle, minimum feret, aspect ratio, round, and solidity as defined in Table 1.

Color Measurement

Seed color was determined using Image J 1.51k software. RGB image represents true color images. The color featured average red (R), green (G) and blue (B) colors. The colors were measured by calculating the mean color parameters of the pixels in the region of interest (ROI). The RGB values were between 0 (no color) and 255 (maximum color).

Grow Out Test

Data were recorded on off-types per plot for each variety and block referred to the passport data of ICRR (Suprihatno et al. 2009) and Standard Evaluation



Fig. 1. Seed purity testing using image analysis for Sintanur (A), and Aek Sibundong (B).

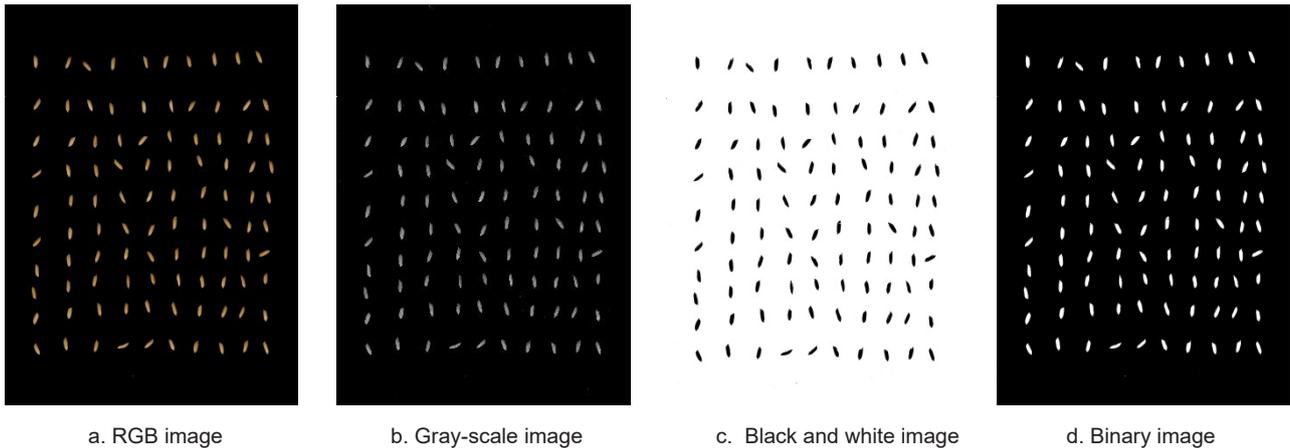


Fig. 2. Red-green-blue (RGB) images of rice seeds resulted from scanning (a) and their conversion using Image J software into gray-scale (b), black and white (c) and binary (d) images.

Table 1. Parameters in Image J software used for morphological trait measurement (Schneider 2012).

Parameters	Definition
Surface area	In calibrated units, such as square millimeters
Perimeter	The length of the outside boundary of the selection (Figure 3)
Length of major axis	The line connecting the pedicel point to awn Note that Image J cannot calculate the major and minor axis length if pixel aspect ratio in set scale (Figure 3)
Length of minor axis	Conversely, was defined as the line perpendicular to the length of the major axis with the longest segment intersecting seed (Figure 3)
Angle	The angle between the primary axis and a line parallel to the x-axis of the image
BX and BY	The coordinates of the upper left corner of the rectangle
Circularity	Value of 1.0 indicated a perfect circle. As the value approaches 0.0, it indicates as increasingly elongated polygon Value may not be valid for very small particles, $\frac{4\pi A}{P^2}$
Feret	Measure of an object size along a specified direction. In general, it can be defined as the distance between the two parallel planes restricting the object perpendicular to that direction
Feret X and Y	Coordinates of the feret diameter
Feret angle	The angle (0-180°) of the feret
Minferet	Minimum caliper diameter
Aspect ratio	An image describes the proportional relationship between its width and its height, $(AR) = \frac{L}{W}$
Round	The inverse of aspect ratio, $\frac{4.A}{\pi L^2}$
Solidity	The texture of the seed surface, $\frac{\text{Area}}{\text{Convex area}}$

System (SES) of IRRI International Rice Research Institute 2013) (Table 2). Percent (%) off type per plot is defined as a number of off types divided by a number of total plants per plot multiplied by 100.

The seed morphological characters were measured based on length, shape (ratio of length to width) and seed color (International Seed Testing Assosiation 2015). Length was measured at maturity growth stage as the distance from the base of the lowermost sterile lemma to the tip (apiculus) of the fertile lemma or palea. For rice variety which possessed awn, the grain was measured to a point comparable to the tip of the apiculus. Width is

measured as the distance across the fertile lemma and the palea at the widest point. Shape is measured as the ratio between length and width. Seed color, especially for brown rice (dehulled grains) is classified into white, light brown, speckled brown, brown, red, variable purple and purple.

Statistical Analysis

Data were analyzed using discriminant analysis using IBM SPSS Statistics 21 software.

RESULTS

Morphology of Aek Sibundong and Sintanur Seeds

Discriminant analysis for determining correlation between variables resulted from Image J of Aek Sibundong or Sintanur seeds showed that only feret X was not significant with their parameters. The correlation values varied among the morphological characters. The smallest value was shown by minimum feret (3%) and the largest was the round (87%) (Table 3).

The values of wilks lamda for these variables were less than 20% indicating that these variables are potentially used as a differentiator ($P \leq 1\%$) (Table 3). The area, minimum feret, feret, aspect ratio, round and solidity of Aek Sibundong and Sintanur seeds were determined using Image J (Table 4). Sintanur showed a larger area than Aek Sibundong. Aek Sibundong ferets were in the range of 0.87–1.09 cm, while those for Sintanur were 0.75–0.97 cm. Round parameter indicated the seed shape. Table 3 shows that Sintanur was more elliptical than Aek Sibundong. The round value showed the highest correlation of 87% (Table 1). The ratio of length and width of Aek Sibundong seed (AR) was 3 – 4.07, corresponded to the description of the variety that Aek Sibundong seed was classified as slender (lean) (≤ 3.0). It was different to Sintanur whose aspect ratio between 2.11 and 2.95.

Variations in area (Figure 4a), feret (Figure 4b), minimum feret (Figure 4c), aspect ratio (AR) (Figure 4d), round (Figure 4e) and solidity (Figure 4f) were observed in this study. Some of Aek Sibundong seed areas were significantly larger than those of Sintanur ($P < 0.001$) (Table 1), except Sintanur11 (Figure 4a). Aek Sibundong seeds were longer in feret and bigger in aspect ratio, but shorter in minimum feret compared to Sintanur. In addition, Sintanur was rounder than Aek Sibundong, for the textural

Table 2. Seed morphological characters of Aek Sibundong and Sintanur rice based on passport data.

Variety	Descriptor	Classification	
		ICRR passport data	SES IRRI
Aek Sibundong	Length	Extra long	(more than 7.50 mm)
	Shape (ratio of length to width)	Slender long	Slender (over 3.0)
	Seed color	Yellow clean	Qualitative character
	Sintanur	Length	Extra long
Sintanur	Shape (ratio of length to width)	Medium	Medium (2.1-3.0)
	Seed Color	Yellow clean	Qualitative character

trait, solidity of Sintanur seeds was significantly larger ($P < 0.001$) than that of Aek Sibundong.

Seed Color

Correlation between color variables resulted from Image J of Aek Sibundong or Sintanur seeds were determined using discriminant analysis (Table 5). Mean red, mean blue and red measurements were significant and correlated with their parameters. The smallest value was shown by red parameter (8%) and the largest was the mean red (75%) (Table 5). The values of wilks lamda for these variables were less than 45% indicating that these variables are potentially used as a differentiator ($P \leq 1\%$) (Table 5).

Distribution of RGB color in Aek Sibundong and Sintanur seeds captured using flatbed scanner (Figure 5) showed that those varieties had different distributions of RGB image. RGB color of Sintanur had a higher value than that of Aek Sibundong (Table 6). A higher RGB color in the seeds indicated a lighter color of the varieties. Differentiation of rice seed colors was difficult using manual projection, therefore, application of Image J provides more precised result in rice seed purity identification.

Figure 6 shows the box plots of RGB color of (a) red, (b) green and (c) blue for Sintanur and Aek Sibundong

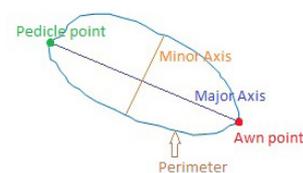


Fig. 3. Parameters used for measuring morphological traits of rice seed.

Table 3. Discriminant analysis of morphological traits of Aek Sibundong and Sintanur rice seeds.

Parameter	Correlation (%)	Wilks Lamda	F value	P value
Area	5	0.99	30.83	≤ 0.001
Perimeter	-37	0.57	1712.68	≤ 0.001
Width	14	0.91	231.24	≤ 0.001
Height	-35	0.59	1582.07	≤ 0.001
Angle	-03	0.99	12.82	≤ 0.001
Circularity	56	0.37	3885.73	≤ 0.001
Feret	-62	0.32	4848.39	≤ 0.001
Feret X	2*	0.99	6.97	0.008
FeretY	-4	0.99	15.52	≤ 0.001
Feret Angle	-4	0.99	15.48	≤ 0.001
Minimum feret	53	0.40	3477.04	≤ 0.001
Aspect ratio	-75	0.24	7032.30	≤ 0.001
Round	87	0.19	9409.95	≤ 0.001
Solidity	24	0.76	706.90	≤ 0.001

* = not correlated

Table 4. Morphological traits of Aek Sibundong and Sintanur rice seeds.

		Area (cm)	Feret (cm)	Minimum feret (cm)	Aspect ratio	Round	Solidity
Aek Sibundong	Min	0.15	0.87	0.23	3.00	0.23	0.87
	Max	0.26	1.09	0.33	4.07	0.35	0.97
	Mean	0.21	0.97	0.29	3.30	0.30	0.95
	St dev	0.02	0.04	0.02	0.28	0.03	0.01
Sintanur	Min	0.18	0.75	0.30	2.11	0.34	0.88
	Max	0.28	0.97	0.40	2.95	0.50	0.97
	Mean	0.22	0.86	0.34	2.46	0.41	0.96
	St dev	0.02	0.04	0.02	0.14	0.02	0.01

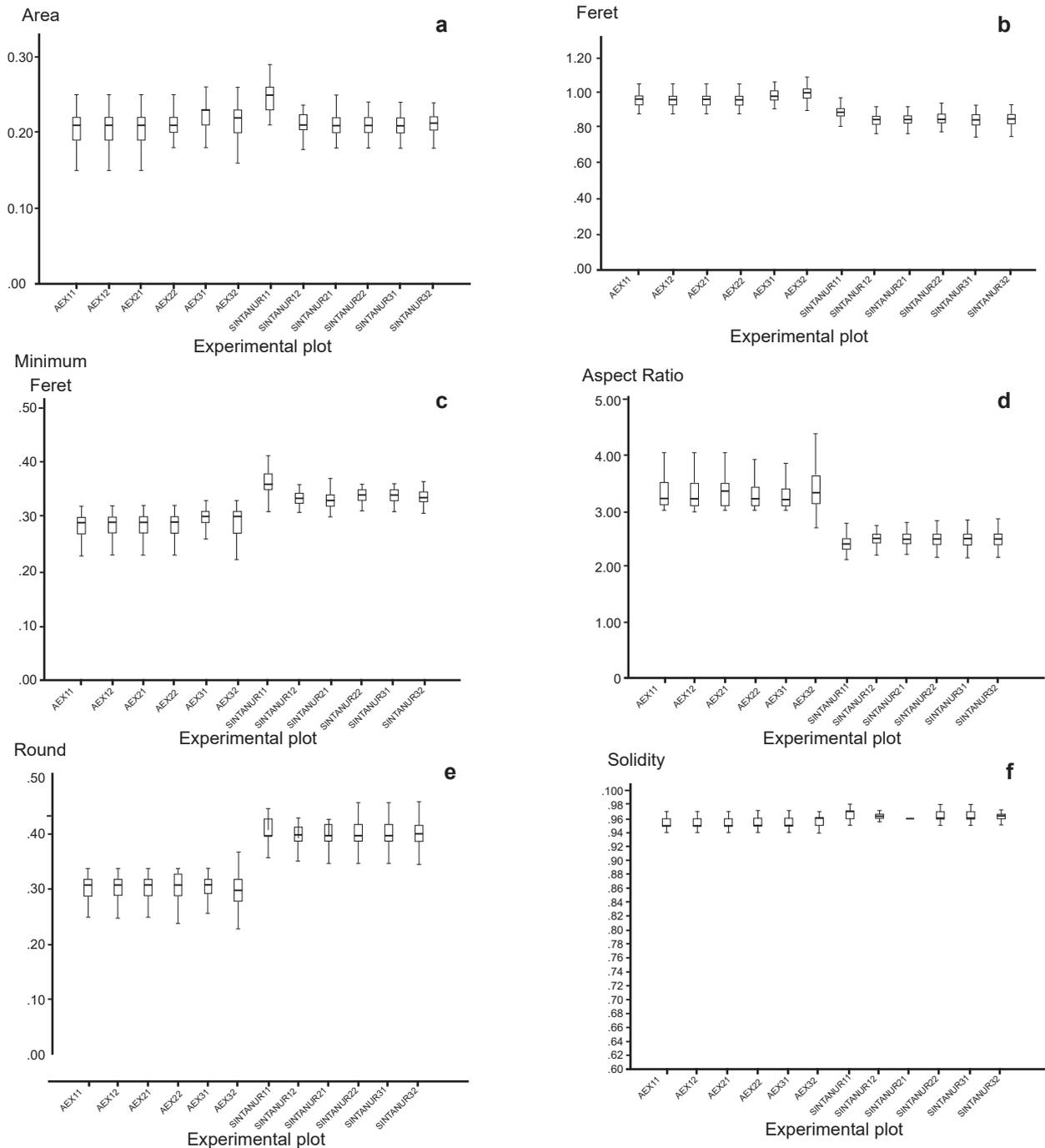


Fig. 4. Boxplots of morphological traits of Aek Sibundong and Sintanur; a = area, b = feret, c = minimum feret, d = aspect ratio, e = round, f = solidity.

seeds. In all color traits, Aek Sibundong seeds showed a larger variation than Sintanur. Off types of rice variety were identified during the generative stage. The off types can be differentiated based on the seed coat color.

Clarification of Seed Purity with GOT

Tabel 7 shows percentage of off types (wrong identification) on purity seed. The recognition rate of the training data set was 98.7% for Sintanur which was lower than GOT, and 99.98% for Aek Sibundong. It was found to be wrong identification as off type seeds were 15 seeds for Sintanur and 4 seeds for Aek Sibundong. The accuracy level of purity test with flatbed scanning was 99.2% for shape and 93.5% for color. Tabel 7 shows that the percent purity seeds of the training data set of Aek Sibundong and Sintanur using flatbed scanning-image analysis were lower than GOT.

DISCUSSION

Nowadays, many image processing tools have been used for image analysis. The study used flatbed scanner because the tool is available in most laboratories, simple, and stable in taking pictures. Clearly this method is different with the camera or microscope which is strongly influenced by external light conditions so it requires special techniques. Generally, image processing techniques are applied for identification, quality control and germination evaluation on seed experiments. In this

study, image processing and analysis techniques were employed for identifying shape and color morphology and also quality control with seed purity test. Seed analysis using flatbed scanner-image analysis takes about 3–10 minutes per sample compared to about 1–2 hours for human vision analysis. The procedure requires a PC with standard desktop scanner commonly found in literally all offices or laboratories. For image analysis and further data processing, a fully automatic procedure was developed using an open source image processing and analysis software and a standard software.

Rice Seed Identification

Indonesia have more than 4.000 rice varieties, and this is a major problem in identifying these varieties. Only expert can recognize or identify many kinds of rice varieties. In the study, six parameters (area, feret, minferet, AR, solidity, and round) were able to describe the rice varieties because the parameters have the correlation value.

Two rice varieties Aek Sibundong and Sintanur, according to SES are classified into the same group as an extra-long seed rice with seed length of more than 0.75 cm (IRRI 2013). By using flatbed scanning method, both varieties can be distinguished quantitatively, the length (Feret) of Aek Sibundong was 0.87–1.0 mm and Sintanur was 0.75–0.97 mm, as well as other parameters for shape and seed color. The seed colors were quantified by measuring the distribution of RGB (red green and blue) colors in rice grains. Kuo et al. (2016) discriminated rice grain by quantifying morphological parameters, texture and color with an accuracy of 89.1%. Adnan et al. (2015) stated that round parameter had a considerable coefficient compared to other parameters, therefore it influenced the mode classified for different rice varieties.

Seed Purity Clarification Using GOT

In this study, both image analyses using flatbed scanning and GOT were conducted to identify off type plants. Thus, research findings can be useful for identification of off type plants which

Table 5. Discriminant analysis color traits of Aek Sibundong and Sintanur rise seeds.

Parameter	Correlation (%)	Wilks lamda	F value	P value
Mean red	-75	0.45	2742.23	≤ 0.001
Mean green	-67a	0.51	2204.54	≤ 0.001
Mean blue	-55	0.92	194.62	≤ 0.001
Digital Number	-20a	0.61	1455.16	≤ 0.001
Green	-17a	0,94	141.61	≤ 0.001
Blue	12a	0.97	66.61	≤ 0.001
Red	-8	0.99	31.37	≤ 0.001

Mean values followed by 'a' letter are not correlated.

Table 6 Seed visualization color of Aek Sibundong and Sintanur rice seeds.

	AS11	A12	AS13	AS14	AS21	AS22	AS23	AS24	AS31	AS32	AS33	AS34
Max												
Min												
	S11	S12	S13	S14	S21	S22	S23	S24	S31	S32	S33	S34
Min												
Max												

AS = Aek Sibundong, S = Sintanur.

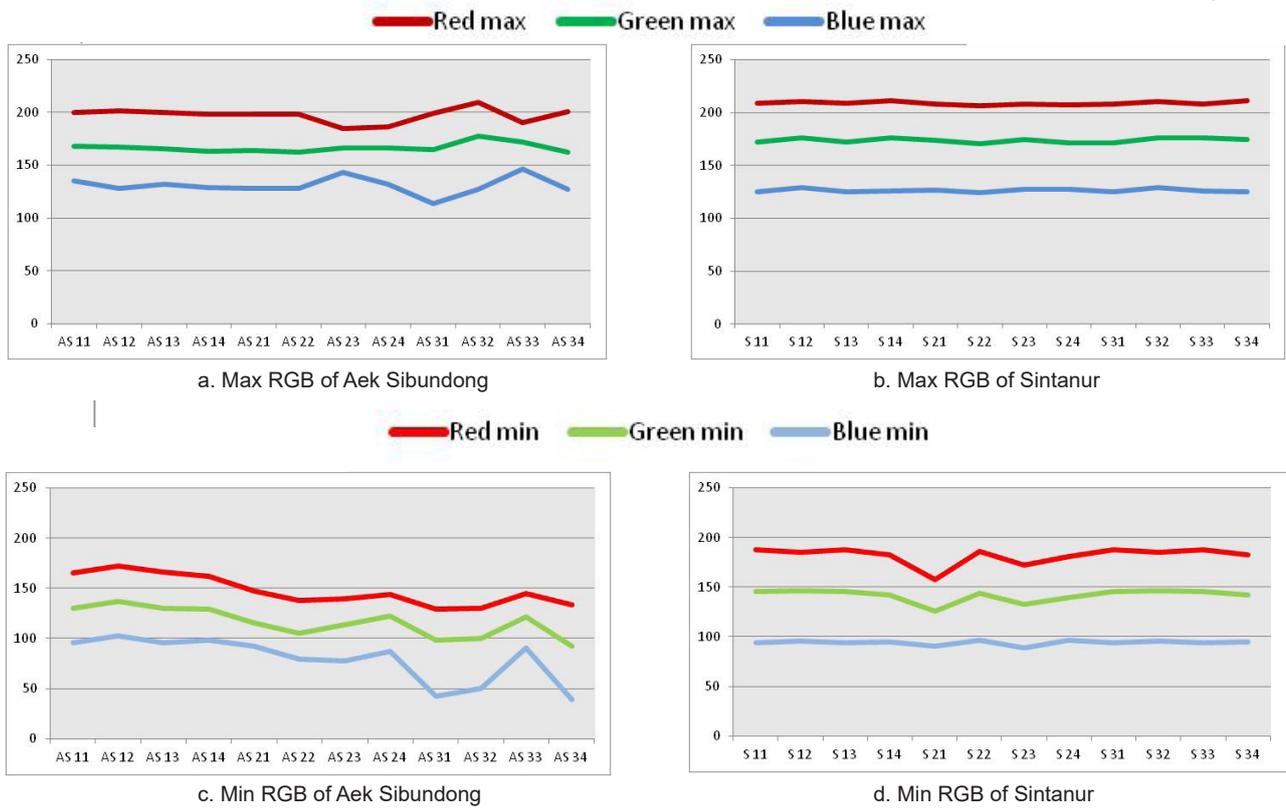


Fig. 5. Distribution of red-green-blue (RGB) color in Aek Sibundong and Sintanur rice seeds captured using flatbed scanner

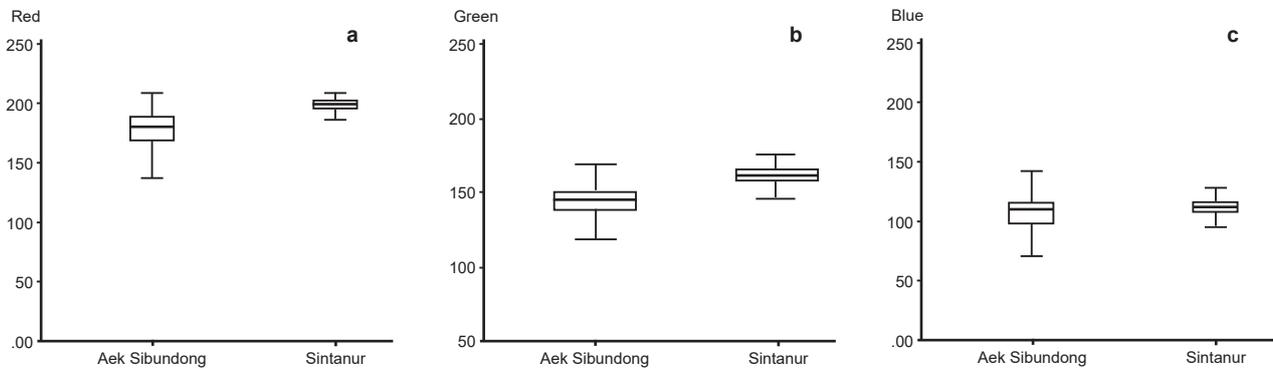


Fig. 6. Boxplots of color traits, (a) Red, (b) Green, (c) Blue.

Table 7. (Percentage) seed purity based on shape and colour compared to GOT.

Variety	Flatbed scanning			Grow Out Test (GOT)	
	Actual seed	True type (%)	Off type (%)	True type (%)	Off type (%)
Shape morphology					
Aek Sibundong	1127	99.60	0.40	99.61	0.39
Sintanur	1142	98.70	1.30	99.98	0.02
	Accuracy 99.2%				
Color morphology					
Aek Sibundong	1127	90.7	9.3	100	0
Sintanur	1142	96.3	4.7	100	0
	Accuracy 93.5%				

cannot be clearly distinguished in the fields. Therefore, an attempt has been made at the ICRR, to determine the off types of Sintanur and Aek Sibudong rice varieties, for maintaining seed purity. A few researchers have measured the morphology of rice seed. Adnan et al (2015) identified rice variety based on area, perimeter, minimum feret, aspect ratio and round and got an accurate value of more than 50%. Others researchers (Kuo et al. 2016) obtained an accurate value of 89%.

In this study, off type plants were calculated by flatbed scanning using image analysis based on shape and color morphology and followed by GOT to confirm the off types by human vision observation in the field. As shown in Table 7, off types with flatbed scanning image analysis were higher than GOT and no off type (100% purity seed) was identified by GOT based on color morphology. So, flatbed scanning and image analysis is more sensitive than GOT.

CONCLUSION

Discrimination of Aek Sibudong and Sintanur rice varieties using image analysis of flatbed scanning was successful with an accurate value of 99.2% for shape and 93.5% for color. Image analysis was more sensitive 1.28% than GOT for Sintanur. This method is faster, cheaper and potential to be used as a complement in rice seed purity testing to increase the accuracy of grow out test. During purity testing, seeds that have the same morphology cannot be identified. Molecular techniques are considered to be a tool to distinguish the seeds.

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