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

Mira Landep Widiastuti\textsuperscript{a}, Aris Hairmansis\textsuperscript{b*}, Endah Retno Palupi\textsuperscript{a} and Satriyas Ilyas\textsuperscript{a}

\textsuperscript{a}Bogor Agricultural University  
Jalan Raya Dramaga, Kampus IPB Dramaga Bogor 16680, West Java, Indonesia  
\textsuperscript{b}Indonesian Center for Rice Research  
Jalan Raya 9 Sukamandi, Subang 41256, West Java, Indonesia  
\textsuperscript{*Corresponding author: satriyas252@gmail.com}

Submitted 26 March 2018; Revised 16 July 2018; Accepted 6 August 2018

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]

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 Saptomo (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 (Ropelew ska and Zapotoczny 2018), measuring pore size distribution of metal foams and porcine trabecular bone (Doktor and Kytýr 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
The seed morphological characters were measured based on length, shape (ratio of length to width) and seed color (International Seed Testing Association 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 ≤ 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 (≤ 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 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 ≤ 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 precise 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

| Table 2. Seed morphological characters of Aek Sibundong and Sintanur rice based on passport data. |
|-----------------|-----------------|-----------------|
| Variety         | Descriptor       | Classification  |
| Aek Sibundong   | Length           | Extra long      |
|                 | Shape (ratio of length to width) | Slender long (more than 7.50 mm) |
|                 | Seed color       | Yellow clean    |
| Sintanur        | Length           | Extra long      |
|                 | Shape (ratio of length to width) | Medium (more than 7.50 mm) |
|                 | Seed color       | Yellow clean    |

| 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
Digital image analysis using flatbed scanning ... (Mira L. Widiastuti et al.)

**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             | 98.7                 | 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 Sibundong 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 Sibundong 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.

ACKNOWLEDGEMENTS

The research was a part of a comprehensive research thesis project. The authors would like to thanks the Indonesian Agency for Agricultural Research and Development (IAARD) for financial support during the first author’s study.

REFERENCES

Adnan, Widiastuti, M.L. & Wahyuni, S. (2015) Identifikasi varietas padi menggunakan pengolahan citra digital dan analisis diskriminan. Penelitian Pertanian Tanaman Pangan. [Online] 34 (2), 89–96. Available from: doi:10.21082/jpptp.v34n2.2015.p.89–96.

Chaugule, A.A. & Mali, S.N. (2016) Identification of paddy varieties based on novel seed angle features. Computers and Electronics in Agriculture. [Online] 123, 415–422. Available from: doi:10.1016/j.compag.2016.03.012.

Doktor, T. & Kytyr, D. (2010) Assessment of pore size distribution using image analysis. Youth symposium on experimental solid mechanics. Trieste, Italy (January), 155–157.

Grassi, S., Casiraghi, E. & Alamprese, C. (2018) Fish fillet authentication by image analysis. Journal of Food Engineering. 234 (16–23), 2018.

Hairmansis, A., Berger, B., Tester, M. & Roy, S.J. (2014) Image-based phenotyping for non-destructive screening of different salinity tolerance traits in rice. Rice. [Online] 7 (1), 1–10. Available from: doi:10.1186/s12284-014-0016-3.

Ilyas, S. (2012) Ilmu dan Teknologi Benih. Bogor, IPB Press.

International Rice Research Institute (2013) Standard Evaluation System (SES) for Rice. Redona, E.D. (ed.) 5th edition. [Online] (June), Los Banos, Philippines, IRRI. Available from: doi:10.1063/1.1522164.

International Seed Testing Association (2015) International rules for seed testing. Switzerland, International Seed Testing Association.

Kaisaat, K., Keawdonnee, N., Chomkoksart, S., Jinntunya, N. & Pattanansiri, B. (2017) Colour measurements of pigmented rice grain using flatbed scanning and image analysis. Journal of Physics: Conference Series. [Online] 901 (1). Available from: doi:10.1088/1742-6596/901/1/012069.

Kuo, T.-Y., Chung, C.-I., Chen, S.-Y., Lin, H.-A. & Kuo, Y.-F. (2016) Identifying rice grains using image analysis and sparse-representation-based classification. Computers and Electronics in Agriculture. [Online] 127, 716–725. Available from: doi:10.1016/j.compag.2016.07.020.

Lurstwut, B. & Pompomanomchai, C. (2017) Image analysis based on color, shape and texture for rice seed (Oryza sativa L.) germination evaluation. Agriculture and Natural Resources. [Online] 51 (5), 383–389. Available from: doi:10.1016/j.jares.2017.12.002.

Maheshwari, C. V (2013) Quality Assessment of Oryza Sativa Ssp Indica ( Rice ) Using Computer Vision. International Journal of Innovative Research in Computer and Communication Engineering. 1 (4), 1107–1115.

Mulsanti, I.W. (2011) Identifikasi dan evaluasi kemurnian genetik benih padi hibrida menggunakan marka mikrosatelite. Institut Pertanian Bogor.

Nurcahyani, A.A. & Saptono, R. (2015) Identifikasi kualitas beras dengan citra digital. Scientific Journal of Informatics. [Online] 2 (1), 63–72. Available from: doi:10.15294/sijit.v2i1.4530.

Ropelewskia, E. & Zapotoczny, P. (2018) Classification of Fusarium-infected and healthy wheat kernels based on features from hyperspectral images and flatbed scanner images: a comparative analysis. European Food Research and Technology. [Online] 0 (0), 1–10. Available from: doi:10.1007/s00127-018-3059-7.

Schneider, C.A., Rasband, W.S. & Eliceiri, K. (2012) NIH Image to ImageJ: 25 years of image analysis. Nature Methods. [Online] 9 (7), 671–675. Available from: doi:10.1038/nmeth.2089.

Suprihatno, B., Daradjat, A.A., Satoto, Baehaki, S.E., Widiarta, I.N., Setyono, A., Indrasari, S.D., Lesmana, O.S. & Sembriring, H. (2009) Deskripsi Varietas Padi. Subang, Balai Besar Penelitian Tanaman Padi. Tielmann, M.R.D. & Hill, T.J. (2018) Air void analyses on asphalt specimens using plane section preparation and image analysis. Journal of Materials in Civil Engineering. 30 (June), 2018.

Zapotoczny, P., Szczypiński, P.M. & Daszkiewicz, T. (2016) Evaluation of the quality of cold meats by computer-assisted image analysis. LWT - Food Science and Technology. [Online] 67 (October 2015), 37–49. Available from: doi:10.1016/j.lwt.2015.11.042.