Thai Society of Agricultural Engineering Journal
Volume 20 No. 2 (2014) 24-32
Available online at www.tsae.asia

Research Paper

ISSN 1685-408X

Quantification of the Severity of Brown Leaf Spot Disease in Cassava using Image Analysis

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Abstract

Accurate assessment of cassava brown leaf spot (BLS) disease severity is needed in epidemic monitoring and plant breeding. The objectives of this study were to develop an image analysis technique for quantifying infection levels of cassava BLS disease, and to compare the assessment results with conventional visual rating using Teri's diagram key. Detached cassava leaves infected by BLS disease of contrasting severity were collected from an experimental field. The leaf images were captured under controlled illumination. The images were pre-processed and read for primary RGB values. An image processing algorithm based on HSI color space has been developed for segmenting lesion region on a leaf using Otsu's method which allows the counting of number of spots (N_s) and the calculation of percentage of infection area (*PI*). Manual scoring has been concurrently carried out by seven human raters using Teri's illustrated diagram which orders BLS infection into 4 levels, representing lesion area of approximately 5, 10, 15 and 20% respectively. The Teri's diagram itself was also scanned and presented to the image processing algorithm developed to investigate actual lesion area. Verification of Teri's diagram indicated that the N_s obtained from image analysis was 100% coincided with that counted by raters. On the other hand, the *PI* values obtained from image analysis were 0.87, 3.94, 9.87 and 18.71%, considerably smaller than visual approximation. Assessment of diseased leaf samples by image analysis and visual rating showed a good agreement for N_s (R^2=0.8993), however, visual rating tended to overestimate the infection level comparing with image analysis, and a large variation among raters could be observed. The results further suggested that the accuracy of spots detection vary proportionally with the infection level. This study has demonstrated the usefulness of image analysis in quantifying cassava BLS disease severity in that it provides more elaborate scaling and better consistency.

Keywords: Cassava, Brown leaf spot disease, Image analysis

1 Introduction

Cassava (*Manihot esculenta* Crantz) is of important economic crop for Thailand which supplies a large consumption as food as well as energy. In 2012, cassava plantation area inside the country has reached 1.48 million ha, giving a total production of 29.8 million tons with an average yield of 0.56 t ha^{-1} (Office of Agricultural Economics, 2013). In the same year, Thailand exported cassava products for 6.96 million tons, achieving an export value of over 2600 million USD (Center for Agricultural Information OAE, 2013).

The effectiveness in cassava production, however, depends substantially on pests and diseases condition during its growing periods. A foliar disease extensively distributed in cassava fields is the brown leaf spot

(BLS) disease caused by a fungal pathogen *Cercosporidium henningsii* Allesch. The symptoms appear as small brown spot within a darker border on the upper leaf surface and a grayish cast on the lower surface due to the presence of conidiophores and conidia. The size of lesion ring ranges from 0.3 to 0.5 mm in width. A yellowish halo surrounding this ring may be found on very susceptible varieties. The necrotic tissue in the center of brown spots may fall, giving a shot hole on the leaf surface (Teri et al., 1978).

The impact of BLS disease is often underestimated because it does not apparently damage the diseased plants, however, infection of the BLS disease can induce leaf chlorosis and premature defoliation which restrict photosynthesis, resulting in yield loss up to 20% (Hillocks and Wydra, 2002; Teri et al., 1980). Furthermore, the infected plants tend to be increasingly susceptible to other diseases. Wydra and Verdier (2002) found a positive correlation between the incidence of cassava anthracnose disease and the occurrence of BLS disease, and some relevance among the BLS disease and white leaf spot disease and root rots. Many research works have consistently demonstrated that the BLS disease is thermophilic and favored by high humidity (Teri et al., 1978; Hillocks and Wydra, 2002; Wydra and Verdier, 2002). This clearly suggested that the BLS disease should not be ignored particularly in the climatic conditions of Thailand.

An accurate and precise assessment of the BLS disease severity is important in various aspects of cassava production. A reliable quantification of infection levels is needed to evaluate the epidemic development of the disease which determines consequent decision in disease management. In plant breeding, scoring of the disease severity is used to measure the resistance of cultivars and hence the accuracy of the evaluation method is very crucial.

The disease assessment is typically performed based on visual estimation by a plant pathologist using an illustrated diagram key developed for a specific pathogen and crop. James (1971) has introduced a series of assessment key for several crops including cereal, forage and field crops. Such diagrammatic scales continue to be developed for different plants until nowadays, e.g. Godoy et al. (1997); Michereff et al. (2009). For cassava BLS disease, Teri et al. (1978) has proposed an assessment key which rates the disease into four ordinal levels based on percentage of necrotic leaf area. Onyeka et al. (2004) rated differently by considering the amount of infection in a whole plant from lower to upper parts rather than by evaluation of each single leaf. These visual rating methods, however, is somewhat subjective since the method relies merely on the discretion of raters, leading to questions about its reliability. Image analysis technique has been introduced as an objective method for analyzing plant diseases severity alternative to conventional method. A systematic experimental scheme conducted by Bock et al. (2008) on citrus canker (*Xanthomonas axonopodis* pv. citri) has demonstrated many superiorities of the use of image analysis software (Assess, American Phytopathological Society, St. Paul, MN) over visual estimation both in terms of accuracy and precision. Similar results were found by Wijekoon et al. (2008) who used Scion Image software, and Bock et al. (2009). Poland and Nelson (2011) observed some effects of rater variability and different rating scales on quantitative disease resistance and, in particular, mapping quantitative trait loci for disease resistance. The optical sensing technique may be applied not only on leaf-by-leaf basis but also to field scale as done by Yang (2010) for bacterial leaf blight (*Xanthomonas oryzae* pv. oryzae) in rice.

A number of research works has suggested the usefulness of using image analysis technique for assessing plant disease severity. However, to our knowledge, the study related to the assessment of cassava BLS disease has not been reported. The objectives of the present study were therefore to develop an image analysis technique for quantifying the infection levels of cassava BLS disease, and to compare the assessment results with visual rating using Teri's diagram key.

2 Materials and Method

2.1 Plant materials

Cassava leaves infected by BLS disease of contrasting severity were collected from an experimental field of Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom, Thailand (Lat 14°2′11″N, Long 99°57′56″E). Major soil properties at the site are given in Table 1. The cassava cultivar was *Rayong 5* which is of medium-resistant cultivar in terms of BLS disease resistance (Kampanich, 2003). The plant age at sampling was six months, at which stage their canopy had fully developed and the plants were naturally infected by BLS disease without systematic inoculation.

Table 1 Soil properties at leaf sampling site.

Property	Value
Texture	Clay loam
pH	7.6
EC_a (dS/m)	0.409
Organic matter (%)	1.82
Phosphorus (mg kg^{-1})	48
Potassium (mg kg^{-1})	60

2.2 Image acquisition

Color images of 48 detached cassava leaves were captured under controlled illumination using a cubical lighting box consisting of four 18W cool daylight bulbs with a color temperature of 6500K mounted on respective top corners of the box, orienting 45° to the camera centerline (Figure 1). Inner surfaces of the box were lined with matte black canvas to minimize undesirable reflectance. The luminance at object position measured by a light meter was 3428 lux in average. A digital camera (Canon, IXY55, Japan) used to acquire the leaf images was installed on the top of the illumination box at a distance of 50 cm above the leaf sample which placed on the bottom with white background. This certain camera distance was selected in order to minimize image distortion while maintaining adequate resolution. The f-stop value was fixed at 3.5 and the focal length was adjusted to 8 mm. The image resolution of 1200×1600 pixel in JPEG format was used. Testing of white balance was performed using a 5×5 cm-piece of white paper as a calibration object. Reading of its RGB intensities indicated values ranged from 222–226 and these values were used as reference. Calibration of scale was done by relating the number of pixels to true size of the calibration object. The size of a sample can later be determined using the relationship established.

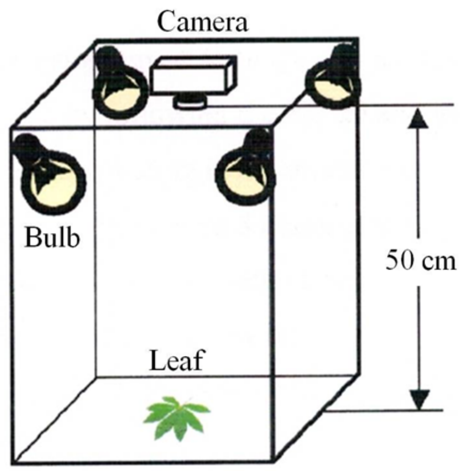

Figure 1 Illumination box for leaf image acquisition.

2.3 Image processing

The Image Processing Toolbox™ for MATLAB® was used in pre-processing and analyzing the images. Each of the original image was resized to 480×640 pixel and read for red (*R*), green (*G*) and blue (*B*) values of each pixel. The image was then converted into HSI color space by calculating hue (*H*), saturation (*S*) and intensity (*I*) values from the chromatic RGB as follows:

$$H = \begin{cases} \theta & \text{if } B \le G \\ 360 - \theta & \text{if } B > G \end{cases} \qquad (1)$$

where

$$\theta = \cos^{-1}\left\{ \frac{\frac{1}{2}\left[(R-G)-(R+B)\right]}{\left[(R-G)^2 + (R-B)(G-B)\right]^{\frac{1}{2}}} \right\} \qquad (2)$$

$$S = 1 - \frac{3}{(R+G+B)}\left[\min(R,G,B)\right] \qquad (3)$$

$$I = \frac{1}{3}\left(R+G+B\right) \qquad (4)$$

In order to calculate the percentage of infection (PI), the total leaf area (A_L) and the diseased area (A_D) must be determined. The total leaf area was segmented from I image using Otsu's method of thresholding as described by McAndrew (2004). The diseased region was extracted from H image by manual thresholding using a predetermined threshold obtained from the tonal histogram. Segmented H image appeared a considerable amount of noise which obstructs the identification of true diseased area. This noise was reduced by opening operation, i.e. the combined erosion and dilation processes described by (Gonzalez and Woods, 2010) using disk-shaped structuring element with a radius of five pixels. The isolated pixels of diseased area were then combined using eight-connected neighborhood criteria, and counted for the number of spots (N_s). Consequently the percentage of infection can be calculated as:

$$PI = \frac{A_D}{A_L} \times 100 \qquad (5)$$

where A_L and A_D were counted in unit pixel. Figure 2 shows the flowchart describing the image processing algorithm. A graphical user interface (GUI) was also developed which allows a user to import an image, observe color space transformation, and displaying the results of the assessment including N_s, PI and infection level (Figure 3).

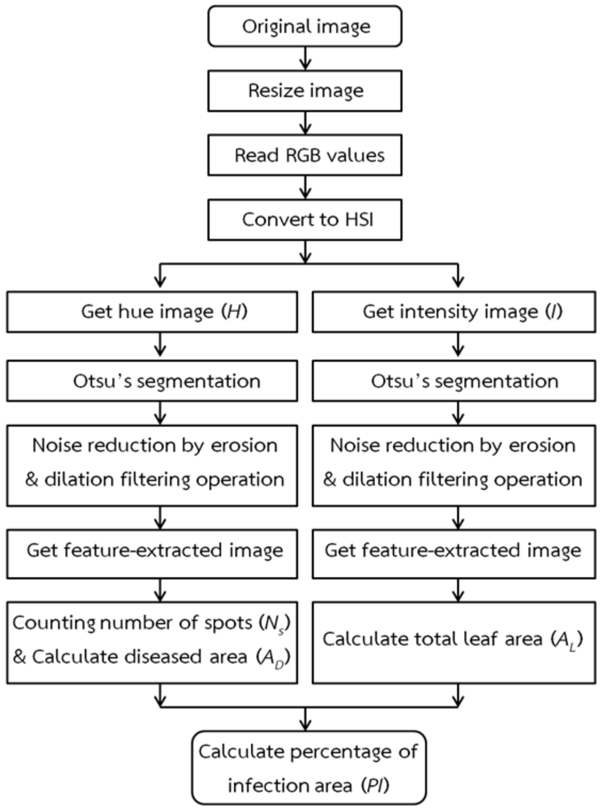

Figure 2 Flowchart of the image analysis algorithm.

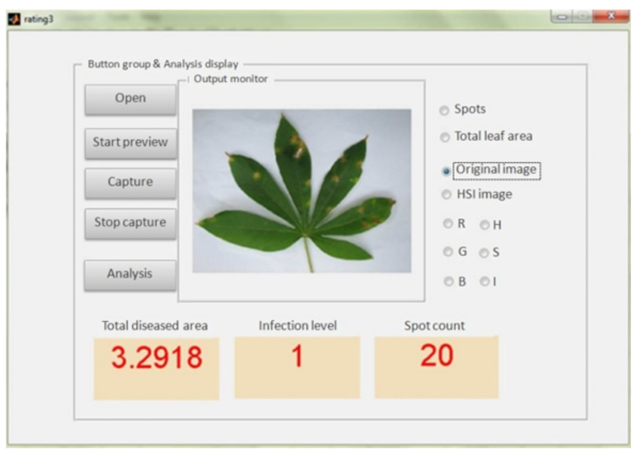

Figure 3 Graphical user interface of the image analysis program.

2.4 Visual rating using Teri's diagram key

Conventional visual assessment of the BLS disease based on Teri's diagram key (Figure 4) has been performed by seven inexperienced human raters. This method classifies the disease severity into four ordinal levels from 1 to 4, corresponds to approximate lesion area of 5, 10, 15 and 20% respectively. The same set of 48 cassava leaf images in random arrangement was presented to each rater along with the diagram key. Since the assessments of the raters were done independently, thus, subsequent results may be attributed to depend merely on the discretion of each individual.

The drawing of Teri's diagram key was then scanned into images and presented to the image analysis process developed in order to investigate actual number of spots (N_s) and percentage of lesion area (*PI*) of each infection level. The results of visual rating including N_s, *PI*, and infection level were compared with those obtainable from the use of image analysis. In addition, an implication of infection level on the accuracy of disease scoring was also observed.

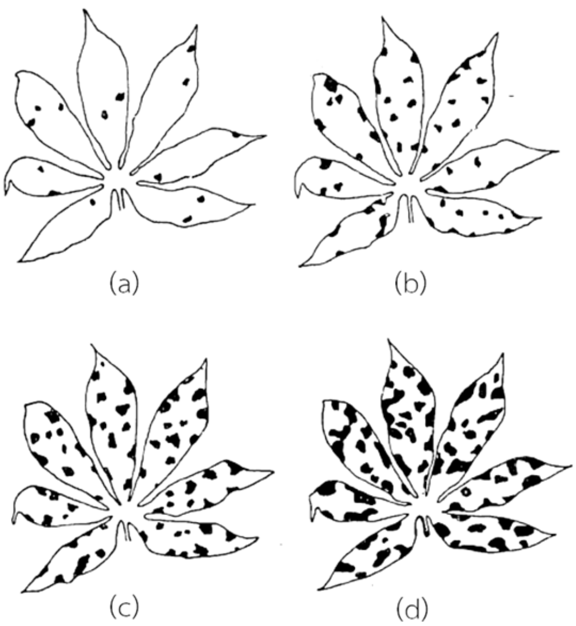

Figure 4 Illustrated diagram key for cassava BLS disease assessment proposed by Teri *et al.* (1978): (a) level 1, (b) level 2, (c) level 3, and (d) level 4.

3 Results and Discussion

3.1 Performance of image segmentation

The pixels of infected region have been extracted from healthy portion based on the difference of *H* value. A key to the success in segmentation has been the determination of a proper threshold value. In this experiment, threshold value was manually selected based on peaks appeared in the histogram. An example of segmented image is illustrated in Figure 5. The total leaf area could be segmented using *I* image associated with Otsu's thresholding method (Figure 5a). Feature of the brown spots has been extracted using *H* image, but with an amount of noise (Figure 5b). However, this noise was successfully eliminated by a combination of erosion and dilation process (Figure 5c).

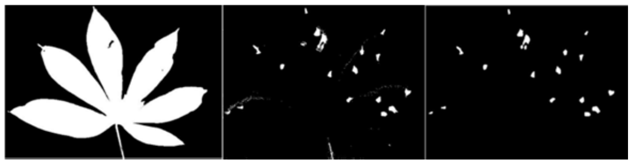

Figure 5 Segmented and feature extracted images: (a) total leaf area, (b) diseased regions with noise, and (c) diseased regions after noise reduction.

3.2 Verification of Teri's diagram

The illustrated diagram of Teri *et al*. (1987) has been imaged an analyzed for N_s and *PI* using the developed image processing algorithm (Figure 6) and compared with the assessment results of human raters. As shown in Table 2, the results showed that the number of spots, N_s detected by image analysis was totally consistent with that counted by raters. However, the percentage of infection area, *PI*, by image analysis was found markedly smaller than visual estimation. In other word, human raters tended to overestimate the lesion area on leaf, which in turn resulting in a probability of misinterpretation of infection level. This result clearly suggests a possibility of inaccurate quantification of the disease due to limitation of human visual perception. It is therefore even more difficult to assess the disease by visual rating if a more elaborate classification, i.e. classifying into more than four levels, is needed. The *PI* based on image analysis was therefore proposed as new criteria and further used in disease scoring in this study.

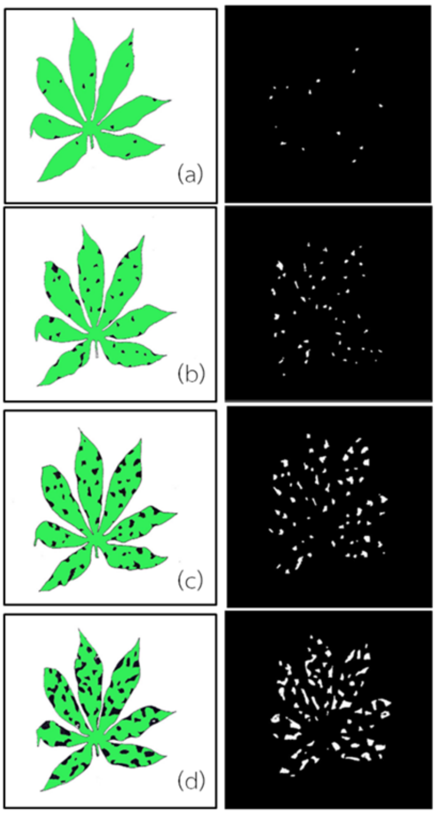

Figure 6 Segmented images of Teri's illustration diagram: (a) level 1, (b) level 2, (b) level 3, and (c) level 4.

Table 2 Verification of Teri's diagram using image analysis.

Infection level	Teri's diagram		Image analysis	
	Number of spots	Infection area	Number of spots	Infection area
1	14	5%	14	0.87%
2	53	10%	53	3.94%
3	65	15%	65	9.87%
4	74	20%	74	18.71%

3.3 Comparison of disease assessment by image analysis and visual rating

Total 48 samples of diseased cassava leaf have been evaluated by image analysis and human raters. The result of number of spot counts, N_s, showed a good correlation (R^2=0.8993) between the two methods (Figure 7). The value of N_s ranged from 2 to 46. Some discrepancies in N_s evaluation might occur when the borders of multiple consecutive spots were not obvious or connected. This misjudgment could be happened in both visual rating and image analysis.

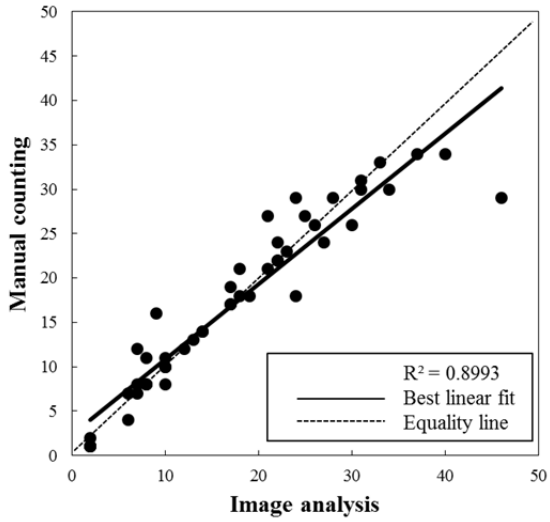

Figure 7 Comparison of number of spot counts assessed by human raters and image analysis.

For severity scoring, in general, most raters found first-level diseased leaves as the majority followed by the second level. Only few raters reported a finding of third-level infection. None of which rated any leaf samples into fourth level (Table 3). The use of image analysis, however, resulted differently in that almost all of the samples were of first-level infection while only one sample was classified into second level. These results suggested the same tendency as discussed previously that human discretion tends to overestimate the disease level so long as Teri's diagram is used.

Table 3 Comparison of disease assessment by image analysis and visual rating.

Disease level	Number of sample							
	Image analysis	Visual rating, by rater No.						
		1	2	3	4	5	6	7
1	47	27	33	41	38	26	32	40
2	1	16	14	7	10	20	15	8
3	0	5	1	0	0	2	1	0
4	0	0	0	0	0	0	0	0
Total	48	48	48	48	48	48	48	48

Variation among raters in disease severity scoring has been observed. Figure 8 shows the mean and associated standard deviation of the severity score of each leaf sample as performed by 7 raters. It is noticeable that the means of seven assessments on most samples were not coincide with the results of image analysis especially in levels 2 and 3. The results further showed a great variance among raters in the assessment which might cause substantial misinterpretation. It seems quite difficult to visually distinguish different patterns of the disease symptom at levels 2 and 3 because the size and distribution characteristics of the brown spots were more or less similar. Experience of a plant pathologist and personal decision are therefore the most important factors influencing the disease severity quantification.

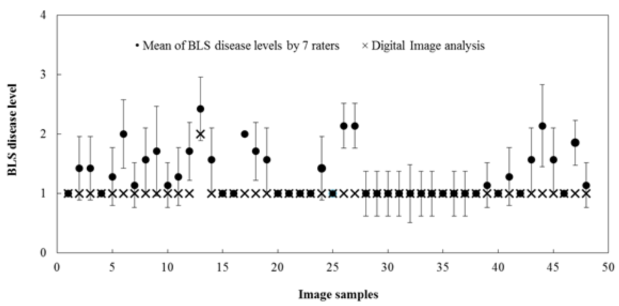

Figure 8 Variation of human raters in disease severity quantification.

3.4 Influence of severity level on the accuracy of image analysis

The result showed some error when the number of spots less than 10. This suggested that the assessment may not be satisfactorily accurate at early stage of disease infection because the size and the number of spot are small. If the spots are small, the erosion and dilation processes might result in an elimination of those spots because they are very similar to the noise. For the leaf sample with more than 10 disease spots, the accuracy of image analysis could be as high as 89.30% and even higher at 91.37% when the number spots was more than 30 spots (Table 4).

Table 4 Influence of number of spots on accuracy of spot detection by image analysis.

Number of spot	Accuracy of spots detection
1 – 10	62.50%
11 – 20	89.30%
21 – 30	88.62%
31 – 40	91.37%
Average	82.94%

In addition to the severity level, the assessment accuracy is likely to have been affected by incompleteness of image pixels. In some instance, multiple spots appeared on an adjacent position and connected to each other, which in turn forming a large circular shape. This type of pixels could mislead the interpretation when using the proposed image analysis procedure. In the opposite, in some instance a single spot contained some hollows inside and appeared as multiple spots (Figure 9). This type of pixels resulted in an overestimation of the spot count. Further improvement of the image processing algorithm is therefore needed to eliminate this shortcoming.

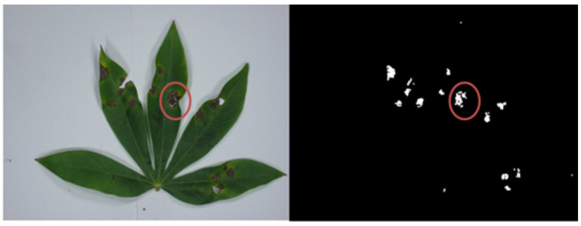

Figure 9 A single spot with inner hollows.

4 Conclusions

A digital image analysis technique has been developed to quantify the severity level of BLS disease infection on a cassava leaf. This technique was capable to estimate the percentage of disease infection defined by lesion area, and number of brown spots. Preliminary verification of conventional visual rating method based on Teri's diagram key has indicated a shortcoming in that it overestimated the percentage of infection area. A new percentage of inflection of the Teri's diagram analyzed by image analysis was therefore used as new criteria for disease assessment. Comparing the results given by human raters further demonstrated many rooms for the occurrence of variance among raters, while the image analysis performed on a certain criteria. The accuracy of image analysis was found increased with number of spots. In conclusion, this study has suggested many advantages of using image analysis technique which helps the plant pathologists as well as plant breeders to perform an accurate scoring of cassava brown leaf spot disease, which in turn enables an agronomist or a farmer to draw countermeasures against the disease and to manage their cassava plantation correctly.

5 Acknowledgement

The present work was financially supported by Kasetsart University Research and Development Institute (KURDI).

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