Quantitative assessment of mosaic disease severity based on digital image processing

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Abstract. Quantitative assessment of plant diseases can be done relatively quickly and practically, especially when applying digital image processing. This paper discusses digital image potential for assessing mosaic symptoms caused by the Tobacco mosaic virus (TMV). Chili plants (Capsicum annuum L.) were grown in pots and subjected to two treatments, i.e., non-inoculated (V0) and TMV inoculated (V1). Plant image recording using a Canon 750D camera with a kit lens was done once a week, starting when the mosaic symptoms are first visible, i.e., in the second-week post-inoculation. Recorded images in RAW format were first converted to TIFF, then subjected to further analysis using image processing applications, namely GIMP 2.8 and Fiji-ImageJ. Differences in the RGB profile of leaves given V0 and V1 treatment was observed. Non-inoculated leaves (V0) have a dark green color pattern, while TMV inoculated leaves (V1) tend to have a mixed color pattern of dark green and bright green. In general, this indicates a decrease in chloroplast's ability to absorb light in diseased leaves, reducing the photosynthesis level. This preliminary experiment shows digital image processing’s potential for estimating the severity of mosaic disease with a high degree of accuracy and precision.

1. Introduction
Assessment of disease severity in the field is generally carried out by observing visual symptoms. Field officers make observations with varying abilities and field experiences. Therefore, this observation method has several weaknesses, including 1) field officers may experience fatigue and loss of concentration, which can reduce the accuracy of the assessment; 2) possible subjective variability of the assessment among field officers; 3) the need for frequent training for field officers to maintain the quality of the assessment; 4) if the further assessment will be carried out in a laboratory, it is necessary to take samples so that they are destructive [1].

The most widely used disease severity assessment is based on several scales, scores, or other qualitative categories. This method can cause inconsistent assessment results because it depends on each field officer's interpretation ability. Therefore, it is necessary to develop a quantitative assessment method that is more objective and relatively easy to do to minimize the possibility of bias in the assessment result. Several publications have reported successful digital image processing-based disease severity assessments [2][3][4][5][6]. Unfortunately, not many studies have reported digital images for symptom assessment of viral infection, such as mosaic symptoms.
The development of reliable methods for assessing the severity of viral symptoms is of great importance. The availability of such methods can be useful in determining the rate of disease progression, evaluating disease control treatments, measuring and predicting future disease, and estimating crop loss [7].

2. Methods

2.1. Plant preparation and virus inoculation
*Tobacco mosaic virus* (TMV) is used as a virus inoculum because the sap is easily inoculated, and mosaic symptoms will obviously be developed. The plant used as the model in this study was the chili plant (*Capsicum annuum* L.) because it was easy to maintain and caused obvious mosaic symptoms when infected by TMV. Plant preparation begins with sowing cultivar Matador on a seedling tray covered with a damp tissue for ± one-week. After germinating, the seeds were transferred to a plastic pot containing a planting medium consisting of a mixture of cow manure, soil, and husk charcoal (1: 1: 1 v / v), 20 pots each for two treatments, i.e., without virus inoculation (V0) and with virus inoculation (V1).

Before and after virus inoculation, all chili plants were kept in insect-free cages placed outdoors and exposed to direct sunlight. Virus inoculation was performed mechanically [8] on plants that have produced at least seven pairs of completely open leaves. TMV inoculum was obtained from Plant Virology Laboratory, Department of Plant Protection, IPB University, and propagated on tobacco plants (*Nicotiana tabacum*).

2.2. Image recording
Image recording using a Canon 750D camera with an EF-S 18-55 mm kit lens was performed between 9:00 – 11:00 am WIB on each plant (V0 and V1) after mosaic symptoms were visible on the virus-inoculated plant. The camera is placed on a tripod at the height of ± 2 m, with the lens positioned perpendicular to the plant canopy's surface.

The camera is controlled by an android smartphone using the Canon Camera Connect application. For the camera to automatically adjust for the ambient lighting quality when performing plant image recording, the camera's shooting mode is set in Aperture-Priority mode (aperture value f/8, auto shutter speed, auto ISO speed). An activating the flash lamp with the white balance set to flash mode. The lens is set at a focal length of 55 mm and uses a one-shot autofocus mode.

2.3. Image processing
CRW (RAW) recording images are converted to TIFF format using the Canon Digital Photo Professional 4 application, and then processed using image processing applications such as GIMP 2.8 and Fiji-ImageJ [9][10][11] with the following stages:

2.3.1. Image pre-processing. Background removal is performed on each plant image (V0 and V1) using GIMP 2.8 application.

2.3.2. Plot simulation. Using the same application (GIMP 2.8), V0 and V1 plant images were randomly arranged on new canvases based on the proportion of symptom severity, i.e., 0% (20 of V0 images), 25% (15 and 5 images for V0 and V1, respectively), 50% (10 images for V0 and V1, respectively), 75% (5 and 15 images for V0 and V1, respectively), and 100% (20 of V1 images) (figure 4). Each symptom severity proportion is arranged on three different canvases as a replication.

2.3.3. RGB image profile. Carry out the RGB profile investigations on asymptomatic (V0) and mosaic-symptomatic (V1) leaf image samples using the menu of *Plugins > RGB profiler* (Laummonerie and Mutterer) in the Fiji-ImageJ application to study damaged and undamaged leaf areas.
2.3.4. **RGB image deconvolution.** Extract the RGB image into separate Red, Green, and Blue channels using the menu of *Image > Color > Colour deconvolution* [12] in the Fiji-ImageJ application.

2.3.5. **Segmentation.** The Blue channel is automatically segmented using the menu of *Image > Adjust > Threshold* (Otsu method) on the Fiji-ImageJ application to separate the light green and dark green areas in the leaf image. The Otsu method is often used in image processing of plant diseases [13][14][15].

2.3.6. **Measurement.** The dark green area (black area) of the binary image (segmented) was measured using the menu of *Analyze > Measurement* on the Fiji-ImageJ application.

2.4. **Statistical analysis**

The mosaic symptom severity assessment method's accuracy and precision were evaluated based on the regression analysis approach and Bland-Altman analysis using add-ins Real Statistic Software release 6.8 [16].

3. **Result and discussion**

3.1. **RGB profile**

Active chlorophyll can be evaluated based on red, green, and blue light reflectance (RGB) because chloroplasts on leaves absorb more red light (630-680 nm) and blue (450-520 nm) for photosynthesis; on the other hand, green light (520-600 nm) is absorbed less. This causes the reflectance of red and blue light to be lower than green light. Using digital camera sensors that produce photographic imagery, all three lights' reflectance can be assessed [17].

Chili leaves, which were not inoculated, showed a dark green color pattern (figure 1), while those inoculated with TMV showed a dark green color pattern mixed with a light green color (figure 2). The dark green area indicates a relatively similar RGB reflectance value, 100 pixels down on average. The composition of green light reflectance values between 50 and 100 pixels on average, while red and blue light reflectance values are below the 50 pixels range.

The presence of bright green areas as a characteristic of mosaic symptoms caused by TMV infection on leaves (figure 2) indicates the composition of different RGB reflectance values, i.e., green and red light reflectance value is above the range of 100 pixels and 50 pixels on average, respectively. Also, the blue light reflectance value indicating the pattern is close to the red light reflectance value.

![Figure 1. RGB profile of chili leaves without TMV inoculation (V0)](image_url)
Generally, this indicates a decrease in chloroplasts' ability to absorb light, which causes red and blue light to be more reflected in TMV inoculated leaves, especially in bright green areas. Likewise, the green light is increasingly reflected in those areas.

3.2. Segmentation

Based on the RGB profile approach, the bright green leaf area is assumed to have been damaged to be used as a basis for determining the severity of the mosaic symptoms. The larger the light green area than the dark green area, the worse the mosaic symptoms. This assumption appears to be consistent with segmentation optimization results using the Otsu method for the two treatments, non-inoculated and inoculated by TMV (figure 3).

Segmentation, starting with the extraction of RGB images into separate R, G, and B channels, shows that only the contrasting blue channel shows the light green area's boundary with the dark green. Therefore, the blue channel was used in the segmentation stage by the Otsu method. Binary-image of the segmentation results performed automatically using the threshold plugin in Fiji-ImageJ indicates that the bright green leaf area will turn white; on the other hand, the dark green area will turn black. The difference in the proportion of the two areas is then used to assess the severity of the mosaic symptoms.

Figure 2. RGB profile of chili leaves inoculated by TMV (V1)
3.3. Assessment simulation

The segmentation applied in the simulation of the symptom severity assessment method in chili peppers organized into five symptom severity levels, namely 0%, 25%, 50%, 75%, and 100% (figure 4), gave the expected results. There was an increase in the proportion of leaf area that was white or considered damaged with increasing severity of symptoms (figure 5).

![Figure 3. Results of extraction of RGB images into R, G, and B channels and segmentation using the Otsu method](image)

![Figure 4. Simulated assessment of different categories of mosaic symptom severity. RGB image (top), binary image (bottom)](image)
Mosaic symptoms on chili leaves were first visible in the second week after inoculation. The area of dark green leaves, which is the most optimal area to absorb red and blue light for photosynthesis, has a major reduction in the severity of symptoms of 25%, 50%, 75%, and 100%. The peak point of reduction in leaf area with dark green color was visible three weeks post-inoculation and then increased again at 4 and 5 weeks post-inoculation (figure 5).

The presence of new leaves that did not show any bright green areas when the image was recorded caused the increasing dark green leaf area in a plant. The new leaves without bright green areas are thought to compensate for the reduction plant's photosynthetic ability due to damage to leaf areas with mosaic symptoms. This is thought to be a resistant response of chili plants to systemic TMV infection.

### 3.4. Accuracy and precision

The weekly assessment of the development of symptom severity formed a non-linear regression pattern, i.e., a quadratic pattern and the prediction plot gave an $R^2$ value of 0.99 (figure 6). The $R^2$ value can be used as a basis for determining the level of accuracy of an assessment method [4], and the high $R^2$ value indicates that this image processing-based symptom severity assessment method has a high degree of accuracy.
Figure 6. The pattern of mosaic symptom severity progression (left) and predictive plots (right)

Figure 7. Bland-Altman analysis plot (α = 5%) for replicates 1, 2, dan 3 (G1, G2, dan G3)

Precision or reproducibility, the extent to which a method of assessment in stable conditions can obtain relatively the same results, is also essential. The Bland-Altman analysis plot (figure 7) indicates that this image processing-based mosaic symptom severity assessment method, when repeated, will provide a precise assessment result. This is indicated by the difference in each group's average value that does not exceed the upper and lower limit values.

4. Conclusion
Viral infection causing mosaic symptoms may affect the reduction of photosynthetic rate. This phenomenon can be studied using digital image processing methods. Furthermore, this method can assess the severity of disease symptoms with high accuracy and precision level.
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