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An effective method of leaf area measurement of sweet potatoes

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Leaf area measurement is one essential methods in the research of agronomy. Many different methods are available for analyzing the plant leaf area and each has its own benefits and draw backs. One commonly used method in the study of leaf area is destructive method using Leaf Area Meter (LAM) in a laboratory. However, due to the limited tools, alternative methods are more employed as these methods are more complicated. Thus, further research is needed to formulate a new method using leaf area estimation. This study analyzed 10 genotypes of sweet potatoes, including Papua Salosa, Antin-1, Antin-2, Antin-2, Beta-1, Beta-2, Sari, Jago, Sawentar and Kidal. The leaf area measurement used is LAM by comparing it with the values of leaf area measurement using rating and Average Leaf Area (ALA) estimation methods. ALA method applying small number of plant samples (<20%) can estimate the leaf area of all experimented sweet potatoes with the value of R^2 = 0.9997 between ALA and LAM resulting in ALA = 0.9985LAM + 11.726. Based on the RMSE value, the ALA method can be used to measure the sweet potato leaf area the age of 60 DAP. In the mechanical method, the value of ALA method is closely similar to that of LAM compared to that of rating method, showing ALA = 0.9998 LAM and rating= 1.1023 LAM.

Keywords: ***Estimation, Morphology, LAM, Leaf Area, Sweet Potatoes.***

INTRODUCTION

Leaves are the main photosynthetic organ in plants, in which a process of converting light energy into chemical energy occurs and accumulates the energy in the form of dry matter(Liu et al., 2005).The plant leaf area shows the mechanism of light radiation interception, transpiration, growth and crop yield. The leaf area is an essential variable for studying plant growth and the interaction between plants and the environment (Gong et al., 2013).In the analysis of plant growth, leaf development becomes a major concern. Non-destructive measurement of the plant leaf area is a highly useful variable in physiological, ecological and agronomic studies (Sala et al., 2015). Among parameters in the leaf characterization, the leaf area and five other parameters which are derived from the leaf area including the Leaf Area Index (LAI), the Net Assimilation Rate (NAR), the Specific Leaf Area (SLA), the Specific Leaf Weight (SLW) and the Leaf Area Duration (LAD) are the most representative parts of the plant and are closely related to environmental factors (Widaryanto and Saitama, 2017). Two important factors to consider in measuring the leaf area are the measurement accuracy and measurement speed. Each factor has its own function. As the measurement accuracy is needed in the measurement of photosynthesis rate and other metabolic processes, the measurement speed is required in measuring the leaf area index (Cristofori et al., 2007).

In general, the measurement of the leaf area can be done either destructively or non destructively or by picking leaves or without picking leaves. Destructive measurement by direct sampling on the plants cannot be conducted repeatedly and even taking one single leaf can potentially affect the experiment treatment (Liu *et al*., 2017). The most commonly used measurement for calculating lthe eaf area is calculating the length and width of the leaf using linear equations. However, it only applies for certain plants (Gong *et al*., 2013). The area constants (c) can be used to determine the individual leaf area of the plant as a correction factor in improving calculus precision (Sala et al., 2015).

The most effective leaf area measurement is the method without picking the leaves as beside the fact that plants are not interfered, this also can be conducted faster and does not require equipment that may be difficult to find.In the rubber plants, the leaf area measurement is done by using the regression equation of the length and width of the leaf. Moreover, in some corps such as corns and soybeans and jackfruit plants, a correction factor is applied on the leaf area from the measurement of the length and width of the leaf(Pierozan Junior and Kawakami, 2013). The optimal number of leaf samples required to obtain sufficient data accuracy is 40-60 leaves (Liu *et al*., 2017). A total of 1,500 leaves are used to obtain an accurate value based on mean error (ME) and root mean square error (RMSE)(Sala et al., 2015). Additionally, regression test using RMSE method is commonly used.(Sala et al., 2015;Kishore et al., 2017).

Research on leaf areas is one essential study, especially in the study of sweet potatoes. The leaf area parameter is directly correlated with the growth parameter especially as one of the parameters in plant growth analysis. A number of obstacles in the leaf area measurement such as having to revoke or destroy the plants thereby requiring addition of the number of plants in the research plot can become a problem in the study. Thus, due to the importance of non-destructive leaf area measurement (without picking the plant leaves and destroying them), this study focuses on the comparison of the estimation to the accuracy of the leaf area measurement (LAM) method.

Sweet potato plant leaves have varied morphological characteristics ranging from sizes, colors and quantities. A study conducted by Saitama *et al.* (2017) shows that the number of sweet potato leaves can reach more than 200 pieces per plant, thus making it difficult to observe the number of leaves. Therefore, this study aims to identify and analyze the use of estimation method with the average leaf area as a method that can be applied in the leaf area measurement of sweet potato plants and compare various methods of leaf area measurement in sweet potato plants to obtain the most efficient method.

MATERIALS AND METHODS

10 sweet potato genotypes were used to develop a leaf area measurement model and four measurements were performed at age 45, 60, 75 and 90 days after planting (DAP) to determine when the leaf area measurement shows the stable results. The sample in each period were 12 sweet potato plants and all leaves which had been perfectly opened in each plant were measured. There were 480 sample plants for 10 genotypes, consisting of Antin-1, Antin-2, Antin-3, Beta-1, Beta-2, Jago, Kidal, Papua Salosa, Sari *and* Sawentar genotypes showing diversity in the leaf edge shapes and sizes. 2 plants in each genotype and each age of observation were used as the plant samples to determine the average leaf area in the estimation method. Therefore, there were 80 plants used in this study to determine the average leaf area. This study was conducted from the end of the dry season in October 2016 until the rainy season in January 2017 in Jatikerto village, Kromengan district, Malang Regency, East Java. Jatikerto is located at an altitude of more than 220 above sea level, with average annual rainfall from 1600 to 5000 mm every year and an average temperature of 16 to 31^{o} C.

The observation methods of the leaf area measurement which were used are as follows:

1. LAM (Leaf Area Meter)

LAM is a tool used to measure leaf area by way of photography (Haryanto, 2015). This method is commonly referred to as the destructive method because the leaves are taken from plants to be scanned on LAM. The steps of this method are as follows:

a. Plant leaf organs to be observed were separated from each sample plant to another.
b. LAM was calibrated, by inserting the A4 paper with the length and width previously measured to obtain the actual area results of the paper. Following that, the paper areawas measured using LAM and then the accuracy of the LAM calculationresult was compared with the actual paper area. Thus, the accuracy percentage of LAM was obtained.

c. The observed leaves were inserted in LAM one by one without interruption until all the leaves hadbeen inserted into the LAM. The LAM values were noted and multiplied by the accuracy percentage.

2. Average Leaf Area (ALA)

This method is performed by measuring the leaf area of the sample plant to estimate the leaf area of the entire plant population. This method aims to facilitate the researcher without destroying all research sample plants used for other observations. Here are the stages to measure the leaf area using EWD method:

a. The plant sample of the leaf area was revoked, and then the number of its leaves (n_s) was counted. The more leaves used in the research, the more accurate the average value obtained.
b. The leaf areas of all obtained leaves were measured by using the LAM method. Thus, the total value of leaf areas (A_s) was obtained.
c. The average value($\overline{A}_s$) of the leaf areas was calculated using the formula as follows:

$$\overline{A}_s = \frac{A_s}{n_s}$$

d. The average value of the leaf area ($\overline{A}_s$) of the sample plants was used to calculate the leaf area of another plant (A_y) by calculating the number of leaves of the another plant (n_y) and multiplying it with the average value of the leaf area ($\overline{A}_s$).

$$A_y = n_y \times \overline{A}_s$$

3. Rating method

This method is conducted by a replica image of the plant. In the leaf area measurement using rating method, the leaves used are still intact and have asymmetrical morphology. The leaves are separated from the main stem and then sorted according to the size. Following that, the leaf with a good shape is taken and a replica of it is made before rated based on 10 log (10A) formula. The value of A is the leaf area resulted from LAM (Sitompul, 2015). The results of this rating will be used as a reference in the field observation. Thus, this method is nearly similar to the measurement method of the correction factor in which the leaf area measurement is conducted in the preliminary method. In the actual observation, the leaf area is matched with the replica result that has been previously made.

In this study, the obtained observation data of the leaf area from 10 genotypes of sweet potatoes with various methods were analyzed by using a correlation test or the r value was searched to determine the closeness between the LAM method and other methods at every age and every genotype. Meanwhile, an R^2test was performed to all data of each genotype to find out the relationship between the LAM method and other methods.

$$Y = a + bx$$

$$a = \frac{\sum Y\left(\sum X^2\right) - \sum X \sum Y}{n\sum X^2 - (\sum X)^2}$$

$$b = \frac{n\sum XY - \sum X \sum XY}{n\sum X^2 - (\sum X)^2}$$

$$R^2 = \frac{\left((n)(\sum XY) - (\sum X)(\sum Y)\right)^2}{(n(\sum X^2) - (\sum X)^2(n(\sum Y)^2) - (\sum Y)^2)}$$

Root Mean Square Error (RMSE) is used to evaluate the linear equations generated between the LAM and the EWD or Rating methods. RMSE is an alternative method for evaluating predicting techniques used to measure the accuracy of prediction of a model.Root Mean Square Error (RMSE) is used to evaluate the linear equations generated between the LAM method and the EWD or Rating method. RMSE is an alternative method for evaluating forecasting techniques used to measure the accuracy of forecasts of a model. The low RMSE value indicates that the variation of the value generated by a prediction model is almost similar to the variation in its obeservation value (Makridakis *et al*., 1982) one of the measurements of error in prediction is Root Mean Square Error (RMSE).

$$RMSE = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}$$

RESULTS

Based on the Figure 1, the morphology and size of the leaves of 10 genotypes of sweet potatoes used to formulate the method of leaf area measurement varies. The leaf shape and morphology significantly affect the leaf area of the plants. The shape of Sari, Beta-1 and Antin-1 leaves is equilateral triangle and that of Beta-2, Antin-2 and Antin-3 forms lobes. As for Kidal, Jago and Sawentar, the leaves have a heart shape and the Papua Salosa species has spear-shaped leaves.

The size of leaves Sari and Beta-2 genotypes is relatively small, while the other eight genotypes have medium-sized leaves. There are 3 types of leaf lobes from the 10 tested genotypes of sweet potatoes, including one, two and three lobes (Table 1)

Table 2 shows that the leaf area average of each genotype at 45, 60, 75 and 90 days after planting(DAP). The size of leaf area of Sari and Beta-2 genotypes are classified as small.
However, the medium size leaf area belongs to Papua Salosa, Jago, Antin-1, Antin-2, Antin-3, Beta-1, Kidal and Sawentar genotypes (Table 2).

DISCUSSION

According to Figure 2, the results of leaf area measurement using rating and ALA methods are closely correlated to that of LAM method. The R^2values of rating and ALA methods toward LAM are 0.9989 and 0.9997 respectively and both have linear relation.The leaf area measurement using rating method tends to be higher compared to LAM method with the equation y=1.098x+38.839 and that using ALA method is lower compared to LAM method with the equation of y=0.9985x+11.726 (Figure 2).

Mathematically, the relation between LAM and ALA method can be formulated as ALA = 0.9985LAM + 11.726 with R^2 = 0.9997 meaning that when LAM is 0, the value of ALA is 11.726 (Figure 3a). On the other hand, in mechanistic analysis, the accepted result should show that if LAM does not indicate any measureable leaf area to be measured (LAM=0), ALA should not also show any leaf area to be measured (ALA=0). Therefore, intercept set can be made with the assumption that if x = 0, y = 0. Thus, the formed relation is shown as ALA = 0.9998 LAM with the same R^2 (Figure 3b)

Mathematically, the correlation between LAM and rating methods rating = 1.098 LAM + 38.839 with R^2 = 0.9989. This means that if LAM is 0, rating values is 38.839 (Figure 4a). However, mechanically, the acceptable result should indicate that if LAM does not indicate any measureable leaf area (LAM=0), rating methodalso should not show any leaf area to be measured (rating=0). Therefore, intercept set can be made with the assumption that if x = 0, y = 0. Thus, the formed correlation is shown as rating= 1.1023 LAM with the same R^2(Figure 4b).

Figure1.Morphology and size of 10 genotype sweet potato leaves

Table1. Morphology and Size of Leaves from 10 Genotypes of Sweet Potatoes.

Genotype	Outline of Leaf	Size of Leaf	Number of Lobes
Sari	Triangular	Small	5
Papua Salosa	Hastate	Medium	5
Beta-1	Triangular	Medium	1
Beta-2	Lobed	Small	5
Antin-1	Triangular	Medium	3
Antin-2	Lobed	Medium	5
Antin-3	Lobed	Medium	3
Kidal	Cordate	Medium	1
Jago	Cordate	Medium	5
Sawentar	Cordate	Medium	1

Table2. Leaf area, number of leaves and leaf area average of 10 genotypes of sweet potatoes

Observation	Genotype							
	Papua Salosa				Kidal			
	45DAP	60DAP	75 DAP	90 DAP	45 DAP	60 DAP	75 DAP	90 DAP
Leaf Area ($cm^2 plant^{-1}$)	4920.70	5955.65	8683.19	10776.23	4218.38	7057.46	9056.68	10686.67
Number of Leaf ($sheet plant^{-1}$)	86.58	103.92	149.00	185.08	102.67	169.50	214.75	251.42
Average of Leaf Area ($cm^2 leaf^{-1}$)	56.83	57.31	58.28	58.22	41.09	41.64	42.17	42.51
Observation	Sawentar				Beta-1			
	45 DAP	60 DAP	75 DAP	90 DAP	45 DAP	60 DAP	75 DAP	90 DAP
Leaf Area ($cm^2 plant^{-1}$)	4921.59	7151.47	8295.01	10784.21	4092.78	9301.38	10487.02	11984.01
Number of Leaf ($sheet plant^{-1}$)	70.25	100.33	115.67	150.25	79.67	174.00	195.42	224.08
Average of Leaf Area ($cm^2 leaf^{-1}$)	70.06	71.28	71.71	71.78	51.37	53.46	53.66	53.48
Observation	Jago				Antin-1			
	45 DAP	60 DAP	75 DAP	90 DAP	45 DAP	60 DAP	75 DAP	90 DAP
Leaf Area ($cm^2 plant^{-1}$)	3881.04	7662.16	10143.70	11939.36	5192.39	7381.39	10152.35	11869.21
Number of Leaf ($sheet plant^{-1}$)	89.25	169.92	222.67	262.75	71.92	100.67	136.83	160.42
Average of Leaf Area ($cm^2 leaf^{-1}$)	43.49	45.09	45.56	45.44	72.20	73.33	74.19	73.99
Observation	Antin-2				Antin-3			
	45 DAP	60 DAP	75 DAP	90 DAP	45 DAP	60 DAP	75 DAP	90 DAP
Leaf Area ($cm^2 plant^{-1}$)	4433.61	9931.68	10428.29	11980.21	4965.59	9303.33	12914.43	12411.19
Number of Leaf ($sheet plant^{-1}$)	80.17	173.17	196.33	209.25	87.42	159.42	219.17	221.25
Average of Leaf Area ($cm^2 leaf^{-1}$)	55.30	57.35	53.12	57.25	56.80	58.36	58.93	56.10
Observation	Sari				Beta-2			
	45 DAP	60 DAP	75 DAP	90 DAP	45 DAP	60 DAP	75 DAP	90 DAP
Leaf Area ($cm^2 plant^{-1}$)	1399.66	3956.21	5227.84	6022.35	2243.42	4491.43	5179.62	7987.47
Number of Leaf ($sheet plant^{-1}$)	63.58	158.75	205.83	238.58	119.08	224.25	256.25	293.50
Average of Leaf Area ($cm^2 leaf^{-1}$)	22.01	24.92	25.40	25.24	18.84	20.03	20.21	27.21

According to figure 3 and 4, it can be seen that the ALA accuracy rate is higher than that of rating, where the value of ALA difference is 0.0002 times lower than LAM, while the difference in rating value is 0.1023 times higher than LAM. In other words, the rounding ALA difference value can be ignored because the value of the rating difference is 511 times more than ALA or ALA difference value is only 0.002 times smaller than rating's value.

According to Table 3,it is seen that R^2values from sweet potato leaf area measurements using linear equation y = a+bx at 45 DAP ranged from 0.79 – 0.99 in 10 genotypes, both using ALA method and Rating method. Generally, at the observation age of 60 DAP, the R^2value from the ALA method reached 1, while the R^2value of Rating method ranged from 0.70 to 0.98. This indicates that the ALA method can be used to measure leaf areasof sweet potatoes faster than the Rating method. Both ALA and Rating methods achieve R^2value of 1at the leaf area observation age of 75-95 DAP. Thus, it shows that both methods can be used to measure the leaf area in which the ALA method can be used earlier.

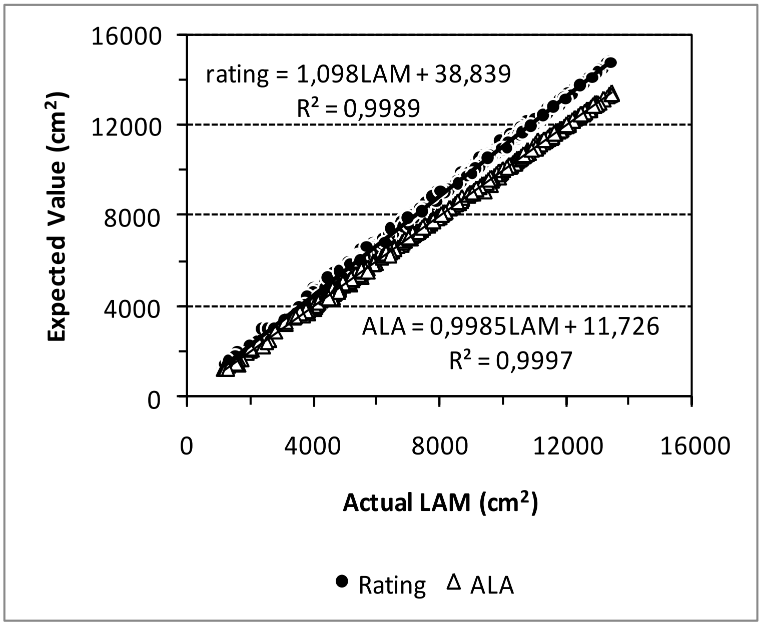

Figure2.Comparison of leaf area measurement using rating and ALA methods

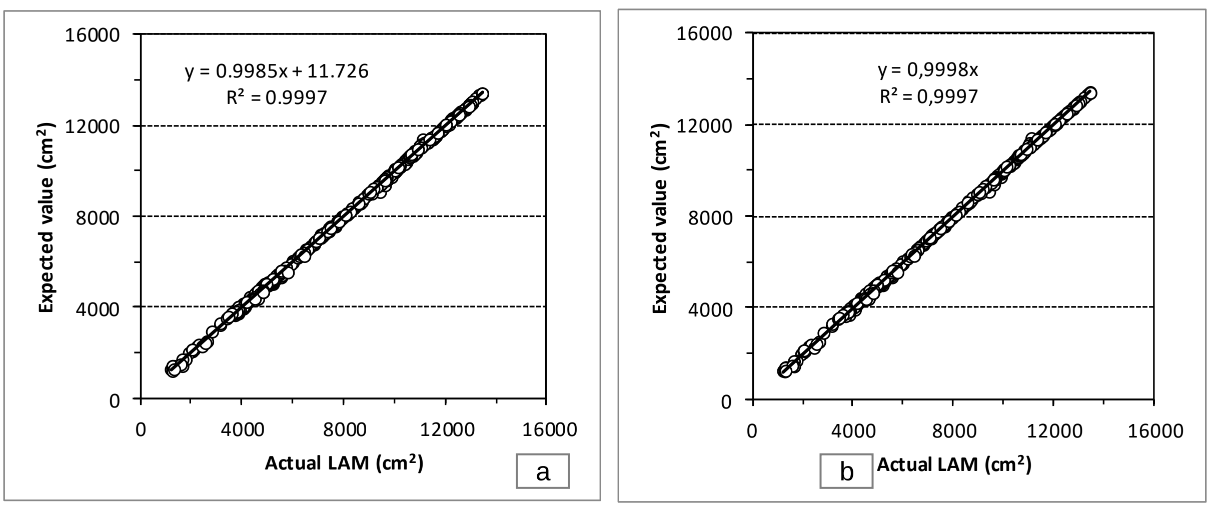

Figure3.Linear regressionobtained between leaf area measurement value of LAM (x) and leaf area measurement value of ALA (y), a) linear equation y = 0.9985x+ 11.726 b) intersep x=0 y=0 with the equation y = 0.9998x

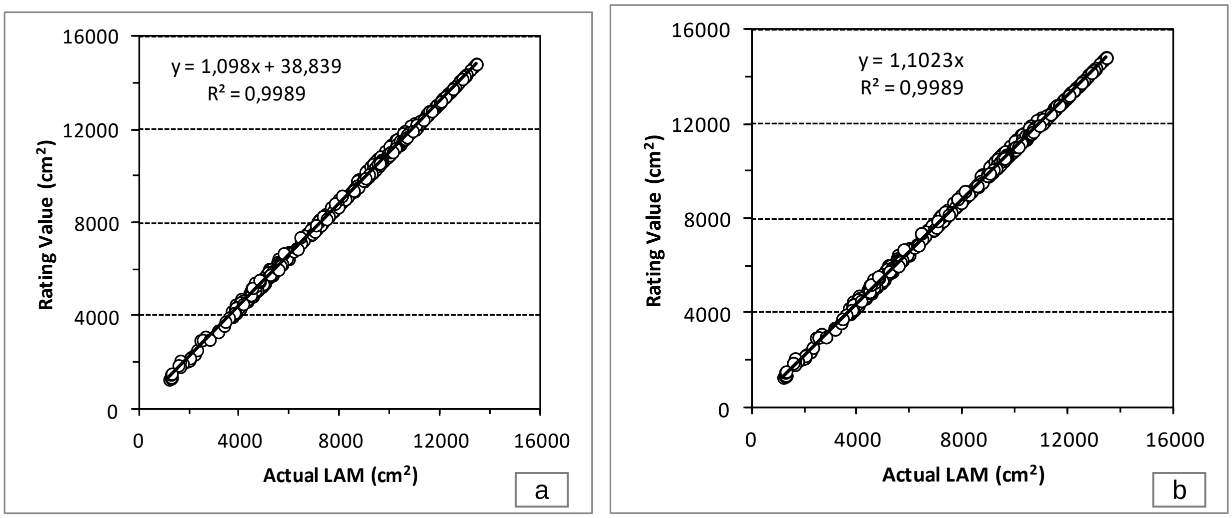

Figure4.Linear Regressionobtained between leaf area measurement value of LAM (x) and leaf area measurement value of rating (y), a) linear equation y = 1.098x+ 38.839 b) intersep x=0 y=0 with the equation y = 1.1023x

Table3. R^2value with equation y = a + bx from each leaf area measurement method of each genotype of sweet potatoes at 4 observation ages : 45, 60, 75 and 90 days after planting (DAP).

Genotip	45 DAP		60 DAP		75 DAP		90 DAP	
	ALA	Rating	ALA	Rating	ALA	Rating	ALA	Rating
	Nilai R^2							
Antin-1	0.98	0.97	0.97	0.92	1.00	1.00	1.00	1.00
Antin-2	0.95	0.90	1.00	0.85	1.00	0.91	1.00	1.00
Antin-3	0.84	0.93	1.00	0.70	1.00	1.00	1.00	1.00
Beta-1	0.95	0.88	1.00	0.86	1.00	1.00	1.00	1.00
Beta-2	0.90	0.96	1.00	0.98	1.00	1.00	1.00	1.00
Jago	0.99	0.99	1.00	0.94	1.00	1.00	1.00	1.00
Kidal	0.96	0.97	1.00	0.93	1.00	1.00	1.00	1.00
Papua Salosa	0.98	0.95	1.00	0.98	1.00	1.00	1.00	1.00
Sari	0.79	0.91	1.00	0.75	1.00	1.00	1.00	1.00
Sawentar	0.97	0.83	1.00	0.93	1.00	1.00	1.00	1.00

Table4. The values of Residual Mean Square Error (RMSE) of each leaf area measurement method for each genotype of sweet potatoes at 4 observation ages: 45, 60, 75 and 90 days after planting (DAP)

Genotip	45 DAP		60 DAP		75 DAP		90 DAP	
	ALA	Rating	ALA	Rating	ALA	Rating	ALA	Rating
	Residual Mean Square Error (RMSE)							
Antin-1	80.90	151.08	82.31	158.80	28.53	4.40	0.24	0.21
Antin-2	79.70	134.76	0.18	186.46	46.41	182.58	0.33	0.30
Antin-3	53.31	94.90	0.11	156.75	0.16	0.01	0.26	0.45
Beta-1	76.82	124.66	0.06	160.54	0.26	0.14	0.46	0.48
Beta-2	59.27	76.82	0.01	67.29	0.09	0.18	0.03	0.33
Jago	52.97	82.32	56.42	136.72	0.29	0.15	1.07	0.50
Kidal	44.06	86.13	0.04	130.15	0.38	0.35	0.40	0.32
Papua Salosa	81.61	144.00	0.08	105.14	0.40	0.23	0.29	0.22
Sari	68.63	84.33	0.11	71.29	0.02	0.31	0.10	0.29
Sawentar	54.35	91.61	0.17	117.43	0.06	0.26	0.02	0.30

Based on Table 4,it is seen that the distribution pattern of RMSE values of both leaf area measurement methods is similar to R^2distribution.

In the measurement of leaf area at the age of 45 DAP, the significantly high RMSE values of ALA and Rating methods show that the linear regression of the relationship between LAM and both methods resulted in a high quadratic value or the equationy = a + bx results in a considerable potential deviation of y.

The RMSE value of the ALA methods was lower than the RMSE value of Rating method on the measurement of leaf area aged 45 DAP of each genotype with the range between 44.06 and 81.90 and 76.82 and 151.08 respectively for ALA and Rating. In general, the measurement of the leaf area on sweet potatoes using the ALA method can be started at 60 DAP, but it does not apply the same for Rating method. At the age of 60 DAP, the RMSE value rating method is considerably large ranging from 67.29 to 186.46. At the observation age of 75 DAP and 90 DAP, the two methods of the leaf area measurement resulted in a low RMSE value of 0.02- 1.07. In some genotypes such as Antin-1, Antin -2, RMSE value was still high until the observation age of 75 DAP. However, the RMSE values of Beta-2 and Sari genotypes tend to be lower than other genotypes.

CONCLUSION

The leaf area measurement of sweet potato leaves using ALA method by observing the sample plants of <20% can be used to estimate the leaf area of all experimental sweet potato plants with ALA = 0.9985 LAM + 11.726 with the value of R^2= 0.9997 and the mechanistic value of ALA = 0.9998 LAM with the same R2. Based on the values of RMSE, the use of ALA method to calculate the leaf area of sweet potato leaves can start at the age of 60 DAP with the RMSE values range 0.70 - 0.98, while at the same age, the RMSE values of rating method range from 67.29 to 186.46. ALA method can be used to estimate the leaf area of sweet potato leaves earlier than rating method. When the mechanistic model is applied, the value of ALA = 0.9998 LAM is closer to LAM than the rating model = 1.1023 LAM.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

Eko Widaryanto : Reviewing and Finalizing the Manuscript. Mochammad Roviq: Data Analysis and Writingthe Manuscript. Akbar Saitama: Collecting and Preparing Data Analysis.

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