

Investigating the Sri Lankan Cassava Mosaic Disease-related Characteristics of Cassava (*Manihot esculenta* Crantz)

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ABSTRACT: In this study, we aimed to investigate which components of cassava (*Manihot esculenta* Crantz) would be associated with the cassava tuber weight when infected with the Sri Lankan Cassava Mosaic Virus (SLCMV). We conducted multiple regressions to find associations between other cassava elements and tuber weights. This study was conducted in the Rukhak Kiri district in Battambang Province, located in northwestern Cambodia, in March 2019. We assessed two major varieties used in the region, namely, KU 50 and Rayong 7, using 90 samples – 60 samples of KU 50 and 30 samples of Rayong 7. The results showed that the tuber weight of KU 50 was heavier than that of Rayong 7. In addition, the biomass of the stem and the weight of all branches and leaves were the sole vital factors associated with tuber weight for both KU 50 and Rayong 7. Moreover, as the tuber number declined, the weight declined rapidly for Rayong 7. Overall, we concluded that SLCMV infection affects cassava's biomass weight.

Keywords: Cambodia cassava, healthy seed, JICA, multiple regression, SLCMD, NUBB.

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INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is one of Cambodia's most important cash crops. Its production has increased rapidly since 2005, reaching 13,512,755 tons in 2019 (MAFF, 2020), with an average yield of 27.20 tons per ha and with Cambodia producing the second highest average yield after Laos in Southeast Asia and the seventh highest in the world (FAO, 2021). Additionally, the total cassava harvesting area in the country in 2019 was 652,531 ha, the second largest area after rice (MAFF, 2020).

Pests and diseases threaten cassava production, such as cassava green mite (*Mononychellus tanajoa*), cassava mealybug

(*Phenacoccus manihoti*), and cassava witches' broom. Furthermore, the Sri Lankan Cassava Mosaic Disease (SLCMD), which is caused by the *Begomovirus* (genus *Begomovirus*, family *Geminiviridae*), has spread rapidly in Cambodia, Vietnam, China, and Thailand (Wang et al., 2016; Uke et al., 2018; Minato et al., 2019; Wang et al., 2019; Leiva et al., 2020; Siriwan et al., 2020; Uke et al., 2021). The vector of the Sri Lankan Cassava Mosaic Virus (SLCMV) is the whitefly, especially *Bemisia tabaci* Asia II 1, which is known to spread *Begomovirus* effectively (Wang et al., 2016). However, human factors also help spread the disease as infected stems are transported across countries (Delaquis et al., 2018).



A preliminary survey in Vietnam reported that the cassava root yield and starch content declined by 16%–33% and 22%–38%, respectively, when SLCMV-infected cuttings were planted, compared to those when healthy disease-free cuttings were used. These findings were consistent with those in Africa (Uke et al., 2021). Another report stated that the crop yield reduction in Zambia due to Cassava Mosaic Disease (CMD) was between 50% and 70% or more (Chikoti et al., 2019). However, The question remains whether there is a significant correlation between cassava root yield and the other parts of the stems, such as the number of leaves or tubers. Therefore, this survey was undertaken to understand the factors associated with the SLCMV-infected cassava yield. The target area was the Rukhak Kiri district in Battambang Province, Cambodia.

MATERIALS AND METHODS

Model

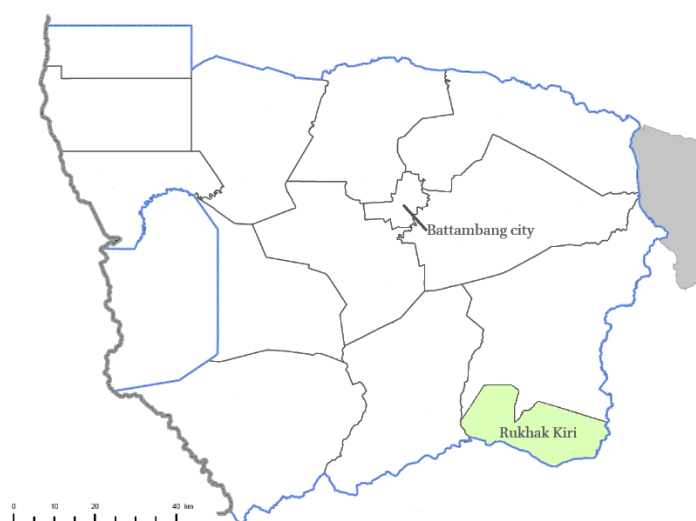
We applied a multiple regression model to determine the association of other cassava elements with tuber weight in addition to the starch content of the infected cassava. Multiple regression is a statistical technique that can analyse the relationship between a single

dependent variable and several independent variables. Multiple regression analysis aims to use independent variables with known values to predict a single dependent value (Greene, 2012). $y_i = \beta_0 + x_{i1}\beta_1 + x_{i2}\beta_2 + \dots + x_{ik}\beta_k + \varepsilon_i, i = 1, \dots, n$

The observed value of y_i is the sum of two parts—aa deterministic and random partscript epsilon sub i.. We aimed to estimate the model's unknown parameters and cassava elements correlated with the ttuberweight calculating the eights beta sub 0, beta sub 1,..., and $\beta_1, \dots, \beta_k$, regression analysis ensures maximal prediction of the dependent variable from the set of independent variables, which is usually done by least-squares estimation.

Study area and sampling procedure

This study was conducted in the Rukhak Kiri district of Battambang Province in northwestern Cambodia (Map 1). Battambang is the largest province, with a total planted cassava area of 108,551 ha and a production of 2,620,638 tons in 2019 (MAFF, 2020). The total cassava harvesting area in the Rukhak Kiri district was 11,937 ha, with an average yield of 26.22 tons per ha, the fourth largest area within 14 districts in the Battambang province in 2020 (BPD AFF, 2021).



Map 1. Rukhak Kiri district, Battambang province, Cambodia (created by the authors based on the Ministry of Planning, 2013).



Fig. 1. Field observation in the Rukhak Kiri district, Battambang province, on the 4th and 8th of March 2019.

On March 4 and 8, 2019, we visited a farm that had SLCMD symptom-like cassava stems in the Rukhak Kiri district and found stunted and distorted shoots and leaves with an evident pale green to yellow mosaic pattern (Fig. 1). The farm manager said that the farm bought stems from a truck from the east side of Cambodia that runs on National Road 5, which reaches Sisophon from Phnum Penhm via Battambang, and planted them several months before. Although we could assume that the stems seemed already affected when planting, the stems the farm bought had no leaves. The diseased stems were unnoticeable because the healthy and diseased stems with the leaves removed were morphologically indistinguishable (Uke et al., 2021). This means stem traders play a significant role in long-distance disease stem dissemination.

Based on their appearance, we determined two varieties, KU 50 and Rayong 7, and collected 90 samples—60 samples of KU 50 and 30 samples of Rayong 7. The mean of the

measured “height of the longest branch among branches of each sample” was 1.572 meters, with a minimum of 0.98 meters and a maximum of 2.12 meters. The mean of the “number of tubers, including tiny ones,” was 7.667, with the minimum number of tubers infected since planting. The mean of the “weight of tubers” was 1.152 kg, with a minimum of 0.290 and a maximum of 2.180. The mean of the “weight of all the branches and leaves (biomass of the stem)” was 0.475 kg, with a maximum of 1.160. The mean of the “number of branches of each sample” was 1.917, with a minimum of one and a maximum of three. Finally, the mean of the “diameter of the stem detached from the root” was 1.747, with a minimum of 1.200 and a maximum of 2.700. A few days after the collection of the leaves and conduction of polymerase chain reaction (PCR) tests, the leaves were determined to be infected by the SLCMV. The planting month for both varieties was June 2018, as they were likely infected since planting.

Table 1. Descriptive summary.

Variables	Definition	Mean	Standard deviation	Minimum (Min)	Maximum (Max)
Dependent variable					
<i>'weighttuber'</i>	Weight of tubers (kg)	1.152	0.454	0.290	2.180
Independent variables					
Variety	(1 = Rayong 7; 0 = KU 50)	0.500	0.504	0	1
<i>'Plant height'</i>	Height of the most extended branch among branches of each sample (m)	1.572	0.319	0.980	2.120
<i>'number'</i>	Number of tubers, including tiny ones (tuber number)	7.667	3.245	2	20
<i>'stemweightall'</i>	Weight of all the branches and leaves (biomass of the stem, kg)	0.475	0.199	0.110	1.160
<i>'numstems'</i>	Number of stem branches (number)	1.917	0.619	1	3
<i>'diameter'</i>	The diameter of the stem detached from the root (cm)	1.747	0.286	1.200	2.700

RESULTS AND DISCUSSION

In this section, we report the regression results presented in Table 2. First, the variable 'plant height' was highly correlated with the variable 'stemweightall' (correlation: 0.817). Thus, we used the variable 'plant height' in Models 1 and 3 and 'stemweightall' in Models 2 and 4. First, we compared the Model 1 of KU 50 and Model 3 of Rayong 7. For KU 50, the plant height did not significantly affect the tuber weight in Model 1. For KU 50, however, the number of tubers, stems, and diameter were significantly correlated with the tuber weight at $p < 0.01$, $p < 0.10$, and $p < 0.01$, respectively. Based on the study above (Uke et al., 2021; Chikoti et al., 2019), when cassava is infected, it is known that the tuber weight declines per stem. Thus, it is likely that the number of tubers, number of stems, and diameter were also associated with tuber weight for KU 50. On the other hand, for Rayong 7, plant height was significantly correlated with tuber weight at $p < 0.10$. The coefficient of plant height indicates that when the plant height is one meter taller, the weight of the tubers will be 0.466 kg more. In addition, the number of tubers and

stems were also associated with the tuber weight for Rayong 7, at $p < 0.10$ and $p < 0.05$, respectively, but not diameter.

Next, we compared the Model 2 of KU 50 and the Model 4 of Rayong 7. The results indicate that the stem biomass, which is the weight of all branches and leaves, is the sole factor significantly associated with the tuber weight for KU 50 and Rayong 7. For KU 50, when the biomass of the stem increased by 1 kg, the tuber weight increased by 1.241 kg. For Rayong 7, when the biomass of the stem increased by 1 kg, the tuber weight increased by 1.539 kg. Finally, the tuber number was statistically correlated with the tuber weight in Model 2 of KU 50; however, there was no other association with the tuber weight for Rayong 7. Finally, the R-squared value indicates a goodness-of-fit measure for the linear regression models. It is a statistical measure representing the proportion of the variance for a dependent variable explained by an independent variable or a variable in a regression model. The highest R-squared value obtained in the study was for the Model 2 of KU 50 (0.639), followed by that of the Model 4 of Rayong 7 (0.625).

Table 2. Regression results.

Variables	KU 50		Rayong 7	
	Model 1	Model 2	Model 3	Model 4
'plant height'	0.418 (0.325)		0.466 * (0.251)	
'numtuber'	0.0772 *** (0.0164)	0.0424 ** (0.0172)	0.0416 * (0.0210)	0.0239 (0.0192)
'numstems'	0.205 * (0.105)	0.100 (0.138)	0.203 ** (0.0954)	0.0322 (0.0996)
'diameter'	0.923 *** (0.226)	0.258 (0.227)	0.178 (0.247)	0.00702 (0.216)
'stemweightall'		1.241 *** (0.377)		1.539 *** (0.428)
Constant	-1.959 *** (0.549)	-0.354 (0.456)	-0.664 (0.419)	0.127 (0.409)
Observations	60	30	30	30
R-squared	0.590	0.639	0.499	0.625

Note: *** $p < 0.01$, ** $p < 0.05$, and * $p < 0.10$, respectively.

Table 3 shows the 95% confidence intervals for the variables. The mean tuber weight KU 50 (1.524 kg/plant) was higher than that of Rayong 7, which had a value of 1.004 kg/plant when infected. The height of KU 50 was also higher than that of Rayong 7, even when infected.

However, the mean number of tubers was more or less equal to seven per plant. The mean stem number was higher in Rayong 7 than in KU 50, but the mean diameter of KU 50 was larger than that of Rayong 7. Finally, the stem weight (biomass weight) of KU 50 was higher than that

of Rayong 7. The 95% confidence interval of the tuber number was between 0.787 and 14.680, with a mean of 7.733 and between 1.361 and 13.506 with a mean of 7.433 for KU 50 and Rayong 7, respectively (Table 3). These results indicate that KU 50 and Rayong 7 will have tuber numbers

between one and 15 once cassava is infected. Using the predicted value from the regression results, we depicted the relationship between tuber weight and tuber number using Models 1 and 3 (Figure 2).

Table 3. The mean and 95% confidence interval for KU 50 and Rayong 7 variables.

Variables	KU 50			Rayong 7		
	Mean	95% confidence interval		Mean	95% confidence interval	
tuberweight	1.524	0.284	2.764	1.004	0.184	1.824
plant height	1.861	1.428	2.293	1.370	0.785	1.954
numtuber	7.733	0.787	14.680	7.433	1.361	13.506
numstems	1.517	0.237	2.796	2.067	0.787	3.346
diameter	1.948	1.324	2.573	1.690	1.100	2.280
stemweightall	0.546	0.153	0.939	0.404	0.049	0.759

Figure 2 shows that when the tuber number decreases to one, it is likely that the tuber weight will decline to 1 kg/plant for both varieties. The decline rate of Rayong 7 was higher than that of KU 50; that is, when the tuber number was 14, the tuber weight was more than 6 kg/plant, and the tuber weight rapidly decreased as the tuber number decreased. On the other hand, when KU 50 was infected, the tuber weight was approximately 2 kg/plant, even when the tuber number was 15.

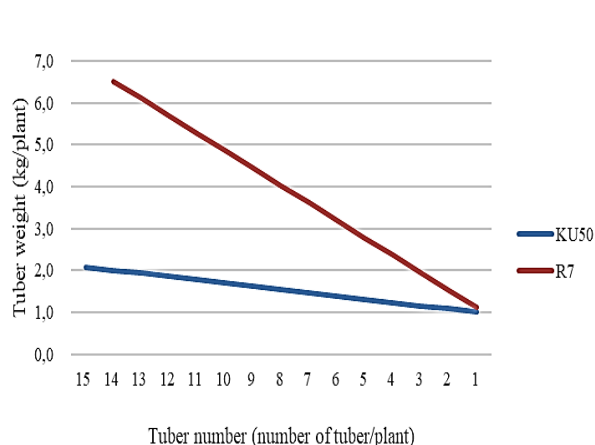


Fig 2. The relationship between tuber weight and number based on the predicted values.

Figure 3 shows the relationship between tuber and biomass weight based on the predicted values. Again, the 95% confidence interval of the biomass weight ("stemweightall") for KU 50 was 0.153–0.939 kg/plant, with a mean of 0.546

kg/plant, and for Rayong 7, it was 0.049–0.759 kg/plant, with a mean of 0.404. These results indicate that KU 50 has a biomass weight of 0.1–1.0 kg/plant once cassava is infected, and Rayong 7 has a biomass weight of 0.05–0.8 kg/plant. When the other variables are equal, and the stem weight of Rayong 7 is more significant, the tuber weight of Rayong 7 is heavier than that of KU 50. The tuber weight declined at a similar rate for both varieties.

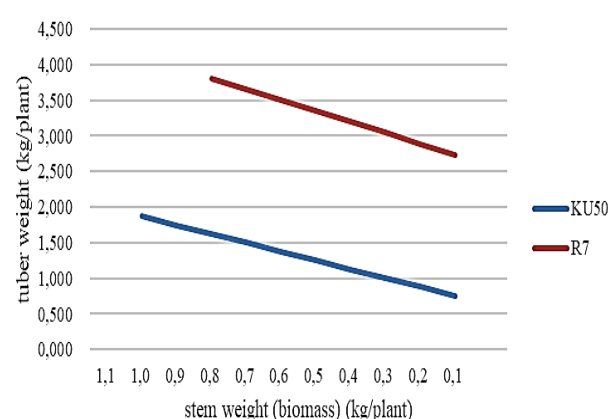


Fig. 3. The relationship between tuber and biomass weight based on the predicted values. R7: Rayong 7.

In this study, we investigated which cassava components were also associated with the cassava tuber weight when infected with the SLCMV. When infected, the mean tuber weight of KU 50 (1.524 kg/plant) was higher than that of Rayong 7 (1.004 kg/plant). In another volume

study, the mean tuber weight of Rayong 7 (3.38 kg/plant) was higher than that of KU 50 (2.75 kg/plant) when cassava was healthy. Although the soil conditions and other environmental factors differed, we cannot simply compare these figures. However, it is interesting that the weight loss was larger for Rayong 7 than for KU 50 when infected. We conducted multiple regression analyses to find associations between other cassava elements and tuber weights. The results indicate that stem biomass, the weight of all branches and leaves, is the sole significant factor associated with the tuber weight for KU 50 and Rayong 7.

CONCLUSION

Thus, we concluded that infection affects biomass weight. The greater the biomass of the stem, the larger the biomass weight is. Similarly, when cassava is infected with the SLCMV, the overall biomass of the stem likely decreases. The variable '*plantheight*' was highly correlated with the variable '*stemweightall*' (correlation: 0.683); however, the variable '*plantheight*' did not have a significant association with the tuber weight of the infected cassava. Finally, as the tuber number declined, the tuber weight declined rapidly for Rayong 7.

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