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ASSESSING THE COCOA GENOTYPES FOR RESISTANCE TO BLACK POD USING THE AREA UNDER THE DISEASE-PROGRESS CURVE (AUDPC)

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Abstract

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In breeding for resistance to black pod, it is important to use an effective and economical method to screen the cocoa genotypes. The detached pod test has been regarded as the most economical and effective screening method as compared to selection of resistant genotypes in the field. This study evaluated the accuracy of the assessment technique used in the detached pod test between the estimation of the area under the disease-progress curve (AUDPC) and the standard assessment of diameter lesion recorded in the sixth day after inoculation of four genotypes at different resistant categories to black pod (KKM4 – susceptible, KKM5 – moderate suscetipble, BR25 – moderate resistant and QH1003 – resistant) at two development pod stages (young and mature pods). The steps involved in the assessment using AUDPC are measured the diameter of the established lesions for six days after inoculation with *Phytophthora palmivora* and calculated the disease severity, fitting the disease progress curve with the nonlinear model, calculated the AUDPC and ranked the cocoa genotypes' resistant based on the AUDPC value. The results indicated that the assessment of the mature cocoa genotypes for resistance to black pod using AUDPC gave better accuracy (100%) of resistant level compared to standard assessment (50%) meanwhile both assessment technique on young pods gave similar percentage (50%) of accuracy. The obtained information will be used as the guide for screening the cocoa genotypes for resistance to black pod.

Key words: area; disease progress; curve; cocoa; detached pod test; nonlinear model; black pod
Abbreviations: AUDPC – area under the disease progress curve

Introduction

Cocoa black pod is the most economically important and widespread disease of cocoa in the world including Malaysia and the losses of cocoa black pod due to *Phytophthora palmivora* were exceeding $400 million worldwide (ICCO, 2013). The symptom caused by *Phytophthora palmivora* began with pod lesions as small, hard, dark spots on any part of the pod, at any stage of pod development (Guest, 2007). Breeding for resistance to black pod has been the most economical, environmentally friendly and effective control method (Iwaro and Singh, 2004) and many research works on developing resistant clones to cocoa black pod have widely carried out. French Agricultural Research Centre for International Development has identified 59 clones proved to be resistant to cocoa black pod with some clones also display qualities in terms of bean characteristics (Thevenin et al., 2012). Barreto et al. (2015) identified

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ten genotypes of cocoa that were resistant to *Phytophthora* disease which was associated with genetic variability. Malaysia has classified 48 cocoa clones planted in Malaysia into four categories based on its resistance to cocoa black pod in the field such as susceptible (5 clones), moderately susceptible (7 clones), moderately resistant (32 clones) and resistant (4 clones) (Haya et al., 2012).

Establishing the effective screening method for selecting the resistant genotypes are important in breeding program. However, selection of resistant plant in the field would take many years as need to wait until a tree bears pods and the infection rates are not always sufficient under natural infection conditions may disrupt the reliability of assessment results (Clias and Despréaux, 2004). Then, the detached pod test was developed to assess differences in resistance between cocoa genotypes to black pod as effectively as possible (Iwaro et al., 1997). The detached pod test identified the potential resistant genotypes to cocoa black pod by comparing the lesion diameter on sixth day after inoculation (DAI) (Iwaro et al., 1997; Clias and Despréaux, 2004; Iwaro et al., 2005; Nyadanu et al., 2009). The advantage of the detached pod test done in the laboratory is able to control the environment and test can be repeated. The test used the lesion diameter to measure the disease severity and assess the cocoa genotypes for resistance to cocoa black pod, however the assessment didn't take into account each day of lesion expansion occurring during the infection in determine the resistant level. Therefore, it is important to consider the change of lesion diameter each day or also known as disease progress curve in assessing the cocoa genotypes for resistance to cocoa black pod.

The work of characterized the disease progress curve with nonlinear models and estimating the area under the disease progress curve (AUDPC) to related the estimated AUDPC to changes in the components of quantitative resistance has been carried out in many other plant diseases such as rice blast disease (Mohapatra et al., 2008), fire blight disease in apple (Momol et al., 1996) and late blight disease in potato (Haynes and Weingartner, 2004) but rarely used in cocoa screening test. Four nonlinear models that commonly used to describe the disease progress curve are the exponential model, monomolecular model, logistic model and Gompertz model (Madden and Campbell, 1990). Thus, this paper was to evaluate the assessment techniques using AUDPC compared to the standard assessment of diameter lesion recorded in the sixth day after inoculation in screening four cocoa genotypes of different resistant categories to black pod at two different pod development stages.

Materials and Methods

Experimental Design

Four cocoa genotypes of different resistant categories such as KKM 5 (susceptible), KKM 4 (moderately susceptible), BR 25 (moderately resistant) and QH1003 (resistant) to black pod (Haya et al., 2012) are selected to evaluate the assessment techniques used in the detached pod test. Two different pod development stages; young pod (less than 3 months old) and mature pod (3 to 4 months old) for each genotype were selected for the assessment in laboratory. The undamaged healthy pods were selected and used in the experiments. The pods were wiped with a piece of cotton wool soaked with 70% ethanol and rinse with sterile distilled water and allowed to air dry for a few minutes.

The detached pod test described by Iwaro et al. (1997) was used in preparing the pod sample for assessment of resistant to black pod. Isolate of *Phytophthora* sp. used in the study was obtained from a naturally infected cocoa pod from a field at the Cocoa Research and Development Center Madai, Sabah, Malaysia. The morphological of the pathogen was confirmed using microscopic observation. A single point inoculation was performed on the ridges of pod. Inoculated pods with mycelial plugs from seven days old *Phytophthora palmivora* culture grown on Potato Dextrose Agar (PDA) were arranged in a randomized complete design (CRD) with five pods per genotype and incubated at room temperature (25±2°C) in the laboratory. The diameter of the established lesions was measured with a caliper meter throughout one to six DAI.

Measuring the disease severity

The assessment to the black pod disease severity was measured by the proportion of lesion size to the pod surface area (Figure 1).

The pod surface was estimated as the prolate spheroid model (Jessop et al., 2010; Ten Hoopen et al., 2012) and the lesion area was estimated based on the ellipse shape (Campbell et al., 2015). The disease severity of the black pod disease was expressed as:

$$y = \frac{\text{The lesion area}}{\text{Pod surface area}} = \\ = \frac{\pi r_1 r_2}{2\pi a^2 + 2\pi a(\frac{ac}{e})\sin^{-1}\left(\sqrt{1-\frac{a^2}{c^2}}\right)} \quad (1)$$

Fitting the disease progress curve

Four nonlinear models were used to describe the disease progress curve or also known as growth-curve models such

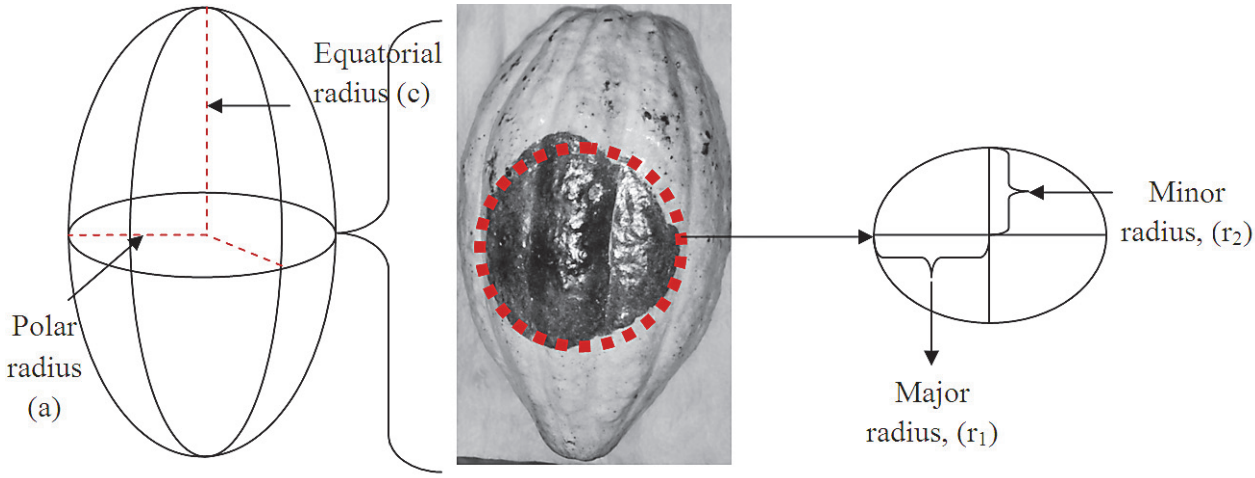

Fig. 1. (a) Prolate spheroid model used to estimate pod surface; (b) Pod with black lesion; and (c) Ellipse model used to estimate lesion area

as exponential model, monomolecular model, logistic model and Gompertz model (Madden and Campbell, 1990; Madden et al., 2007). Table 1 summarized the nonlinear models for disease progress curve.

Statistical Analysis

Curve fitting on the black pod disease severity was done using PROC NLIN using numerical method of Levenburg-Marquardt to minimize the error sum of squares of fitted models (SAS Institute, 2011).

In order to identify the best fitted model among the four nonlinear models, two goodness of fit tests were used, namely the Akaike Information Criterion (AIC) (Burnham and Anderson, 2003) and the Bayesian Information Criterion (BIC), (Schwarz, 1978). The best model was selected based on the smallest value of AIC and BIC. The mathematical expression for both tests was given as follows;

$$AIC = 2p - 2\ln(L) \quad (2)$$

$$BIC = p\ln(n) - 2\ln(L) \quad (3)$$

where: p = number of parameters, n = sample size and $\ln(L)$ = maximum log-likelihood of the estimated model and calculated as follows:

$$\ln(L) = 0.5 \times [-N \times (\ln(2\pi) + + 1 - (\ln(N) + \ln(\sum_{i=1}^{n} x_i^2))] \quad (4)$$

where: $x_1, \ldots, x_n$ = the residuals from the nonlinear least squares fit and N = their number.

Estimating the Area Under Disease Progress Curve

The area under disease progress curve (AUDPC) was calculated for each nonlinear model fitted to the disease progress curve using the method of Yeh (2002) based on the trapezoidal rule that approximate the area under a curve

Table 1
Summary of differential and integrated equations for common growth curve models used in plant disease epidemiology

Model	The rate of disease progress, $\frac{dy}{dt}$	The disease progress model, y	Estimated parameters
Exponential	$\frac{dy}{dt} = r_E y$	$y = y_0 \exp(r_E t)$	y_0 is the initial disease intensity r_E, r_M, r_L and r_G are the rate parameter (constant)
Monomolecular	$\frac{dy}{dt} = r_M(1-y)$	$y = 1 - (1 - y_0)\exp(-r_M t)$	
Logistic	$\frac{dy}{dt} = r_L y(1-y)$	$y = \frac{1}{1 + \exp\left(-\ln\left[\frac{y_0}{1-y_0}\right] - r_L t\right)}$	
Gompertz	$\frac{dy}{dt} = r_G y[\ln(1) - \ln(y)]$	$y = \exp[\ln(y_0) \times \exp(-r_G t)]$	

by dividing the area into a number of strips of equal width. Then, the sum of approximate area of each strip by the area of the trapezium formed will give the approximation of area under the curve. The trapezoidal rule can be presented as integral function given as follows:

$$\int_a^b f(x)dx \approx T(a, b, n) = \\ = ((b - a) / n) \times (((f(a) + f(b)) / 2) + \\ + \Sigma f(a + i (b - a) / n)) \quad (5)$$

Where the domain [a, b] of the integration function are subdivided into n strips with the points of: $x_0, x_1, \ldots, x_n$. $x_0 = a$, $x_n = b$ and $x_r = x_0 + r (b - a) / n$.

In our case of study, $a = 1$, $b = 6$ and n is set to 50 subdivisions or strips while the $f(x)$ is given as nonlinear function model of each clone.

Results

The selection of different pod development stages used in this study varied in pod length based on genotypes (see Table 2). The size of the young pod used in the study ranged between 124.00 mm to 179.00 mm in length, 188.40 mm to 207.00 mm in perimeter and 20 793.21 mm^2 to 30 756.15 mm^2 in estimated pod surface. The mature pod has the range of 159.00 mm to 233.00 mm in length, 235.00 mm to 284.00 mm in perimeter and 36 509.39 mm^2 to 44 782.38 mm^2 in estimated pod surface.

The progress curves of mean lesion diameter and disease severity are shown in Figure 2 with the curves observed in each genotype was progress differently. The assessment using the mean lesion diameter on young pod separated the genotypes very clearly compared to assessment of disease severity. Both assessments used in mature pod show the same trend of disease progress curve. The genotypes of BR25, QH1003 and KKM5 progress slowly compared to KKM4 at the young pod. We can observe that the slope of the disease progress curves at the mature pod increased rapidly compared to young pod.

The overall F values were significant ($P<0.05$) in all developed models except monomelcular model in QH1003 indicated the model's fit were excellent. In general, disease progress fitted well within the Gompertz and logistic models ($P<0.01$) with the smallest AIC and BIC values. At young pod, clone QH1003 and BR25 the Gompertz model was the best to describe disease progress, and the logistic model was the best for the genotypes KKM4 and KKM5 (Table 3). At mature pod, the disease progress in most of the genotypes well described by Gompertz model.

Most of the initial disease (y_0) observed are almost zero and the slope of the Gompertz line (Gompertz rate = r_G) is estimated between 0.3372 to 0.6454 for young pod and 0.2625 to 0.3549 for mature pod.

The used of Gompertz model in estimating the AUDPC of the genotypes for resistance to black pod using young pod showed both assessments only able to match correctly two genotypes (QH1003 and KKM4) for the resistant ranking given in the field assessment (Table 4). However, the assessment using AUDPC was performed better than the mean lesion diameter at sixth DAI on the mature pod as the assessment with AUDPC able to match 100% correctly the resistant ranking to the field assessment on black pod. The value of AUDPC estimated varied among genotypes for mature pod with QH1003 (1.4815), BR25 (2.5064), KKM5 (2.7156) and KKM4 (3.5248).

Discussion

A reliable detached pod test in screening resistant genotypes is important in breeding program. Understanding the pod phenology is an important factor in determine the resistant level to the disease as Soria (1974) has reviewed majority resistant clones fall within the cacao groups of Amazon and Trinitario. The result in Table 2 showed resistant

Table 2
Summary of pod phenology for four cocoa genotypes at young and mature pods

Pod development stage	Genotype	N	Mean ± Standard error		
			Length (mm)	Perimeter (mm)	Pod surface area (mm^2)
Young	BR25	5	179.00±7.14	207.00±6.63	30 756.15±2 156.56
	QH1003	5	162.00±12.41	188.40±2.62	25 299.30±2 030.59
	KKM5	5	124.00±7.31	193.80±11.04	20 793.21±2 363.99
	KKM4	5	163.00±4.06	196.80±8.74	26 710.85±1 787.60
Mature	BR25	5	181.00±8.72	252.00±5.83	38 515.01±2 497.90
	QH1003	5	233.00±3.39	235.00±3.16	44 782.38±1 236.05
	KKM5	5	159.00±2.92	266.00±4.05	36 509.39±1 151.96
	KKM4	5	170.00±15.41	284.00±9.48	42 095.30±4 689.80

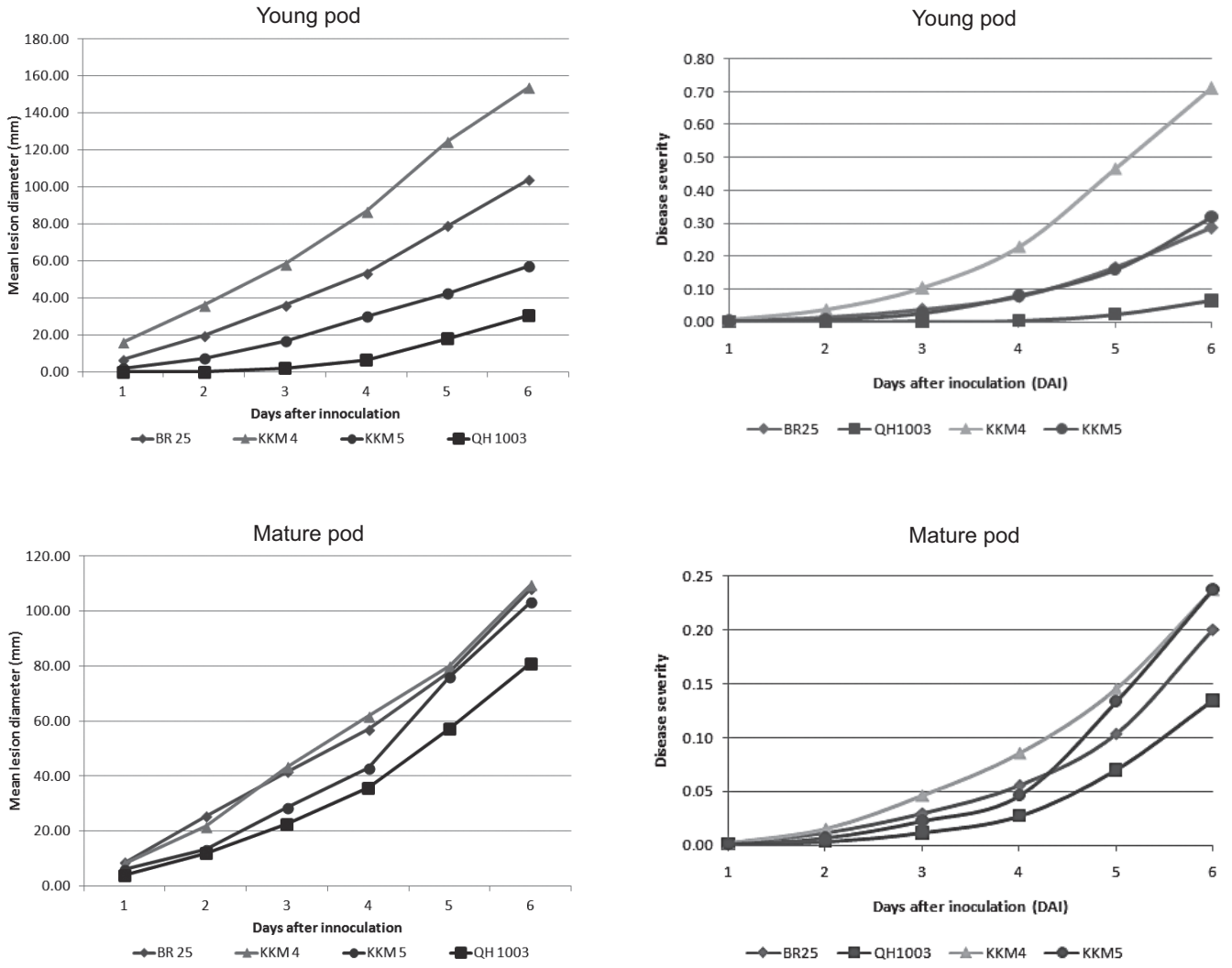

Fig. 2. Mean lesion diameter and disease severity on four genotypes measured on six consecutive days after inoculation at young and mature pods

genotype has different physical characteristics compared to susceptible genotypes. The resistant genotype QH1003 has the longest pod length but small in perimeter indicated very thin in pod shape meanwhile genotypes BR25, KKM4 and KKM5 are in group of Amelonado with a smooth pod surface, shallow furrows and slight bottle neck with larger in pod perimeter but shorter in pod length (Wood and Lass, 2001). Therefore, the study showed it is important to estimate disease severity of black pod by the proportion of lesion size to the pod surface area.

The results in disease progress curve fitting given in Table 3 suggested Gompertz model fitted well to the curve and used to estimate the AUDPC. The findings also supported by Plaut (1980) and Berger (1981) who reported that Gompertz model was better statistical fit to other statistical models in other plant diseases including estimation of epidemic rate, projection of future disease severity and determination of initial disease. The estimated slope of the Gompertz line for young pod was higher than mature pod in Table 3 indicated the Gompertz model projected that *Phytophthora palmivora* spreads rapidly in young pod compared to mature pod. This could be due to the differences among cocoa pod stages in biochemical contents such as carbohydrates (soluble and insoluble sugars) where young pod contained less carbohydrates compared to mature pod where Nyadanu et al. (2013) reported that less susceptible cocoa genotypes contain more

Table 3
The Goodness of fit test for four nonlinear models on disease severity

Model	Clone	y_0	r	F value	AIC	BIC
Young Pod						
Gompertz	BR25	0.000051	0.3431	2241.61**	-44.74	-45.15
Logistic	BR25	0.00378	0.7798	1318.14**	-41.56	-41.97
Exponential	BR25	0.00578	0.6533	507.45**	-35.84	-36.26
Monomolecular	BR25	-0.1046	0.0588	21.76**	-17.45	-17.87
Logistic	KKM4	0.00469	1.0433	18071.60**	-45.80	-46.22
Gompertz	KKM4	1.19E-08	0.6454	450.43**	-23.68	-24.09
Exponential	KKM4	0.0219	0.5856	235.73**	-19.82	-20.23
Monomolecular	KKM4	-0.3002	0.1715	20.13**	-5.57	-5.99
Logistic	KKM5	0.00208	0.9027	1748.58**	-42.45	-42.87
Exponential	KKM5	0.00369	0.7446	769.82**	-37.54	-37.95
Gompertz	KKM5	1.43E-06	0.4076	759.57**	-37.46	-37.87
Monomolecular	KKM5	-0.1212	0.0638	15.21*	-14.72	-15.13
Gompertz	QH1003	8.33E-10	0.3372	3318.79**	-66.74	-67.16
Logistic	QH1003	0.000045	1.2203	624.15**	-56.73	-57.15
Exponential	QH1003	0.000053	1.1817	565.26**	-56.14	-56.56
Monomolecular	QH1003	-0.0236	0.0109	6.22ns	-30.73	-31.15
Mature Pod						
Logistic	BR25	0.0029	0.7423	2197.98**	-49.12	-49.54
Exponential	BR25	0.00379	0.6618	1968.66**	-48.46	-48.88
Gompertz	BR25	0.000125	0.2841	474.96**	-39.95	-40.37
Monomolecular	BR25	-0.0668	0.0389	22.15**	-22.05	-22.47
Gompertz	KKM4	0.000898	0.2625	2271.62**	-46.58	-46.99
Logistic	KKM4	0.00669	0.6412	781.49**	-40.18	-40.60
Exponential	KKM4	0.00861	0.5557	454.69**	-36.95	-37.36
Monomolecular	KKM4	-0.0788	0.0492	42.05**	-22.91	-23.33
Gompertz	KKM5	5.82E-06	0.3549	1071.57**	-42.82	-43.24
Logistic	KKM5	0.00197	0.00197	494.75**	-38.20	-38.61
Exponential	KKM5	0.00298	0.7335	309.31**	-35.39	-35.81
Monomolecular	KKM5	-0.0892	0.0474	15.45*	-18.10	-18.52
Gompertz	QH1003	0.000017	0.2827	5087.89**	-59.23	-59.65
Logistic	QH1003	0.00114	0.8200	986.02**	-49.40	-49.81
Exponential	QH1003	0.00142	0.7597	668.35**	-47.07	-47.49
Monomolecular	QH1003	-0.0487	0.0258	15.63*	-25.24	-25.66

Note: d.f. – Degree of freedom, * – significant at 5% level, ** – significant at 1% level and ns – not significant at 5% level

carbohydrates than the highly susceptible genotypes.

The results of comparing two assessment techniques in Table 4 showed both assessments on young pod only able to match 50% the ranking of resistant categories based on the field assessment as main reference in this study. For the mature pod assessment, the AUDPC gave 100% accuracy of the resistant categories ranking similar to the field assessment compared to the mean lesion diameter only gave 50% accuracy. This suggests mature pod to be used in screening the genotypes for resistance to black pod as the development of mature pod is more stable compared to the young pod and able to give the repeatability and reliability of the detached pod Test. The suggestion also supported by Kebe et al. (2006) in his work to used mature pod at four months old in order to provide reliable information in detached pod test. Besides that, application of AUDPC in assessing the geno-

Table 4
Comparing two different assessment techniques used in the detached pod test for resistance to black pod

Genotypes[1]	Assessment using Mean lesion diameter (mm) at sixth DAI[2]		Assessment using AUDPC[3]	
		Young pod		
QH1003 (R)	30.6 mm	(R)	0.4682	(R)
BR25 (MR)	104.0 mm	(MS)	3.6356	(MS)
KKM5 (MS)	57.0 mm	(MR)	3.5748	(MR)
KKM4 (S)	153.8 mm	(S)	9.8669	(S)
		Mature pod		
QH1003 (R)	80.9 mm	(R)	1.4815	(R)
BR25 (MR)	108.0 mm	(MS)	2.5064	(MR)
KKM5 (MS)	103.3 mm	(MR)	2.7156	(MS)
KKM4 (S)	109.6 mm	(S)	3.5248	(S)

Note: [1] indicated the resistant categories (R – resistant, MR – moderately resistant, MS – moderately susceptible and S – susceptible) based on field assessment; [2] indicated the resistant categories (R – resistant, MR – moderately resistant, MS – moderately susceptible and S – susceptible) based on ascending order for mean lesion diameter;[3] indicated the resistant categories (R – resistant, MR – moderately resistant, MS – moderately susceptible and S – susceptible) based on ascending order for AUDPC

types for resistance to black pod will give better result compared to mean lesion diameter at sixth DAI.

Conclusions

In conclusion, it is practically recommended to use the AUDPC in assessing the mature genotypes for resistance to black pod as it has proved to be effective in terms of reliable and repeatable test compared to the assessment using mean lesion diameter at sixth DAI. The protocol assessment suggested in this study are measured the disease severity as the proportion of lesion size to the pod surface area, then fitting the disease progress curve with Gompertz model, followed by estimating the AUDPC from the developed model and rank the resistant level from resistant to susceptible based on the ascending value of AUDPC.

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