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Qualifying and Quantifying Basal Stem Rot (BSR) Disease Using UV/Vis Diffuse Reflectance Spectroscopy

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ABSTRACT

Basal Stem Rot (BSR) disease was considered as the most destructive disease in oil palm tree. Ganoderma boninense. fungi causing BSR on oil palm tree, could release cell wall degrading enzymes (CWDE) which responsible to degrade polysaccharide on oil palm tree cell wall such as cellulose and lignin into reducing sugar. In this research optical measurement using UV/Vis Diffuse Reflectance Spectroscopy (DRS) was utilized to qualify and quantify BSR level based on its reducing sugar. Several stages were conducted to qualify and quantify reducing sugar comprising sample preparation, spectral data acquisition and chemometrics analysis. Health Stem (HS) and Infected Stem (IS) were prepared. The two stem condition were dried and powdered separately then mixed into 5 mixtures from both stems (100% HS, 75% HS + 25% IS + 50% HS + 50% IS, 25% HS +75% IS and 100% IS) and duplicated to produce 10 total sample. Reducing sugar was measured for each sample. Other than that, spectrum acquisition data was conducted using UV/Vis DRS. In the final stage, chemometrics analysis was performed where reducing sugar was set as response and spectral data was set as predictor. The result showed that Principal Component Analysis (PCA) could well classify 4 group of mixtures. While for quantitative analysis, Partial Least Square (PLS) and Support Vector Machine Regresion (SVR) were used to develop prediction model of reducing sugar. PLS showed low performance with the highest R^2 val and R^2 cal accounting for 0.218 and 0.019 and RMSEC and RMSECV were 0.019 and 0.023, respectively. Besides, SVMR using first derivative savitzky-golay (DG1) preprocessing showed high R^2 cal and R^2 val accounting for 0.823 and 0.701 with RMSEC and RMSECV were 0.012 and 0.028, respectively.

Keywords: Basal Stem Rot, reducing sugar, UV/Vis DRS, chemometrics

ABSTRAK

Penyakit Busuk Pangkal Batang (BPB) merupakan salah satu penyakit tanaman yang cukup mematikan bagi tanaman kelapa sawit. Penyakit tersebut disebabkan oleh cendawan Ganoderma boninense yang dapat memproduksi enzim pengurai dinding sel (CWDE) yang dapat mendegradasi komponen polisakarida pada tanaman sawit menjadi gula reduksi. Pada riset ini, dilakukan pengukuran optic dengan UV/Vis *Diffuse Reflectance Spectroscopy* (DRS) untuk analilis kualitas dan kuantitas pada level penjangkitan BPB berdasarkan nilai gula reduksi. Beberapa tahap dilakukan mulai dari preparasi sampel, akuisisi data spektrum dan analisis chemometrics. Batang sehat (HS) dan Batang terinfeksi (IS) dikeringkan dan digiling hingga menjadi bubuk. Dua jenis sampel tersebut kemudian dicampur menjadi 5 campuran (100% HS, 75% HS + 25% IS + 50% HS + 50% IS, 25% HS +75% IS dan 100% IS) dan diduplikasi sehingga didapat 10 data. Gula reduksi diuji dan data spektrum diuji pada setiap sampel. Selanjutnya, dilakukan analisis chemometrics dengan data gula reduksi sebagai respon dan data spektrum sebagai prediktor. Hasil menunjukkan bahwa *Principal Component Analysis* (PCA) dapat mengklasifikasi 4 campuran dengan baik. Sedangkan untuk analisis kuantitaf, digunakan *Partial Least Square* (PLS) dan *Support Vector Machine Regresion* (SVR) untuk membentuk model prediksi gula reduksi. PLS menunjukkan hasil yang rendah dengan nilai koefisien determinasi tertinggi yang dapat dihasilkan adalah sebesar 0.218 and 0.019 untuk masing-masing R^2 val and R^2 cal serta RMSEC dan RMSECV sebesar 0.019 dan 0.023. Akan tetapi, SVR dengan pra-prosesing first derivative savitzky-golay (DG1) menunjukkan hasil yang baik dengan R^2 cal dan R^2 val sebesar 0.823 dan 0.701 serta RMSEC and RMSECV sebesar 0.012 dan 0.028.

Kata kunci: Penyakit Busuk Pangkal Batang, gula reduksi, UV/Vis DRS, *chemometrics*

INTRODUCTION

Oil palm plantation is a prominent asset in Indonesia that made up around 6.78 million ha followed by Malaysia (5.24 million ha) and Thailand (0.71 million ha), making Indonesia has the largest oil palm plantation worldwide (FAO, 2020). Based on statistics, global palm oil consumption experienced an increase in the last five years up to 75.4 million metric tons (Statista, 2021) and it is projected that the demand of palm oil will grow over the year. In order to satisfy the global demand, Indonesian oil palm industry has to increase and optimize all aspect not only in production plant but also in the field.

However, oil palm plantation is currently face serious issue that could decrease the productivity. Basal Stem Rot (BSR) disease is considered to be a major disease that lower the production of Fresh Fruit Bunch (FFB) oil palm as a raw material to produce CPO (Crude Palm Oil). There is an evident that the disease could lower CPO production up to 35% (Lisnawita, 2016). BSR is a disease that attack oil palm tree caused by soil-borne pathogen called Ganoderma boninese (Siddiqui, 2021). This fungi, Ganoderma boninese, can directly spread to host plant through root-to-root contact, basidiospore and free secondary inoculum in the soil. The fungi slowly infect the oil palm tree without any symptoms and after 60-70% of infection causing at least half of cross-sectional area of stem destroyed, the external symptoms will emerge (Chong, 2017). Therefore, the farmer could only identify the disease in severe stage to death based on the emergence of symptoms such as white rot fungi on the plant (Yen, 2021), unopened spears of oil palm tree (Chong, 2017; Yu, 2018), and stem rooting (Kamu, 2015). As a result, the production and the size of FFB will decrease. Thus, the disease is categorized as the most destructive disease in palm oil plantation (Siddiqui, 2021; Yen, 2021). Therefore, it is important to study the technology that could be applied to do early detection for BSR.

Some studies reveal that Ganoderma produces cell wall degrading enzymes (CWDE) such as amylase and delignification enzyme to break plant cell wall (Chong, 2017 Ress, 2006). When infecting, CWDE from Ganoderma is responsible to break plant components such as cellulose and lignin and converted into reducing sugar (Wang, 2020). Other than that, UV/Vis Diffuse reflectance spectroscopy has shown its capability to predict reducing sugar (Silva, 2018) which is potential to detect BSR. Yet, there is no research on the use of DR UV/Vis spectroscopy to determine the presence of BSR. So, it is interesting to explore more on it.

Some studies propose to prevent the spread of BSR by using early detection system such as imagery classification (Santoso, 2018; Hashim, 2021), terrestrial laser scanning (Husin, 2020) and e-nose (Kresnawaty, 2020). All these research confirming that researcher still discover the most effective method to overcome the spread the disease.

In this paper, the research focuses on the use of UV/Vis Diffuse Reflectance Spectroscopy to identify the BSR. The research may provide other perspective to detect BSR based on reducing sugar. And more, chemometrics analysis was carried out for developing prediction model. It is expected that the method could be a basis study to do further research on developing detection system using spectrum analysis on field in order to prevent the spread of disease in Indonesian oil palm plantation.

METHODOLOGY

Sample Preparation

In this study, two different oil palm stems (healthy stem (HS) and infected stem (IS)) were taken from local plantation in Jember, Indonesia. The two samples were then dried and powdered in separate containers. All the preparation was conducted in Mechatronics Laboratory, Department of Agricultural Engineering, Universitas Brawijaya. Then, the powder is sieved to separate the coarse powder to produce fine powder. Two different fine powders were mixed into 5 different mixtures (100% HS, 75% HS+25% IS, 50% HS+50% IS, 75% HS+25% IS and 100% IS) and each was conducted in duplicate, so that, there were 10 samples in total.

Spectrum Acquisition

Spectral acquisition was conducted in Science and Technology B.J Habibe Laboratory, Maulana Malik Ibrahim State Islamic University. UV/Vis Spectral data of sample were obtained using UV/Vis DRS type Evolution 22, Thermo Scientific ranging from 200 to 800 nm. The data were utilized as predictor to predict reduction sugar. UV/Vis DRS was chosen as it is better utilized for dull surface material (Francis, 1998).

Reducing Sugar Measurement

Reducing sugar was measured using dinitrosalicylic acid (DNS) method (Miller, 1959), glucose standard solution was made by measuring λ max using UV/Vis spectrometer. The standard reducing sugar curve was made in several stages, namely the manufacture of glucose standard solutions with concentrations of 100 ppm, 200 ppm, 400 ppm, 600 ppm, 800 ppm, and 1000 ppm, then the glucose standard solution was added with 3,5-Dinitrosalicylic acid (DNS) reagent and KNa-Tartrate 40%. The absorbance was measured using a UV-Vis spectrophotometer at a maximum wavelength of 540 nm. The final stage is the determination of reducing sugar in sawdust by obtaining an absorbance value that will be made for a standard curve and calculated using the equation, so that the calculation of reducing sugar content in the sample is expressed in units of mg/g. The standard curve was made (Figure 2). Then reducing sugar was calculated using Equation 1.

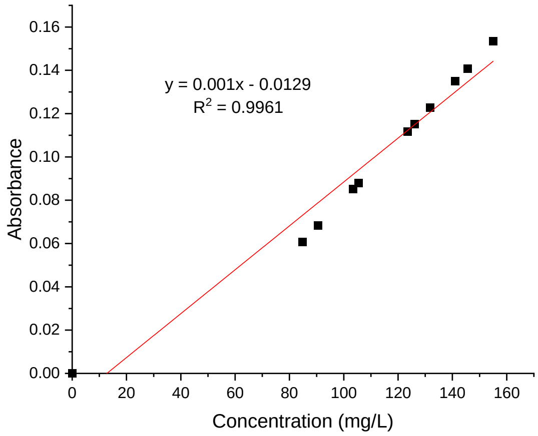

Figure 1. Standard curve of reducing sugar

$$RS = \frac{C*V}{m} \quad (1)$$

where:

RS = Reducing sugar (mg/g)
C = Sugar concentration on the sample (mg/L)
V = Volume of sample extract (L)
m = Sample weight (g)

Chemomectric Analysis

Chemomectrics was performed using Unscrambler X 10.3 Trial version. Principal Component Analysis (PCA) was run to classify 5 mixtures. Partial Least Square (PLS) and Support Vector Machine Regression (SVR) analysis were performed to quantify reduction sugar. Several pre-processing techniques were conducted to improve the spectral signal such as: First Derivative Savitszky-Golay (DG1), Standard Normal Variance (SNV), Multiplicative Scattering Correction (MSC) and normalization (Rinnan, 2009). Determination Coefficient (R^2) and error (RMSEC and RMSECV) were examined to understand the performance of model (Iqbal, 2020; Chanda, 2019).

RESULT AND DISCUSSION

Level of Reducing Sugar

According to the study of Yu, 2018, at final stage of ganoderma infection, the level of polysaccharide was not found, which means that the enzymatic process was completely converted polysaccharide (cellulose and lignin) into reducing sugar. Therefore, the decrease of lignin and cellulose were followed by the increase of reducing sugar level. However, measuring reducing sugar on 5 mixtures of health and infected oil palm trunk did not show an increase trend which might be caused by certain conditions.

Maillard process during dying also contributed to the modification of reducing sugar, a study by (Lund, 2017) confirmed that there was a condensation of reducing sugar at first stage of maillard and it can lead to the decrease of it. Some Ganoderma species has been observed to delignify wood selectively (Ress, 2006), which might affect reducing sugar on infected sample creating no trend on mixture sample. Moreover, while fully infected oil palm trunk was expected to have high level of reducing sugar, the sample showed the different. So, mixing certain concentration of health and infected oil palm trunk did not represent the actual reducing sugar on BSR infection stage.

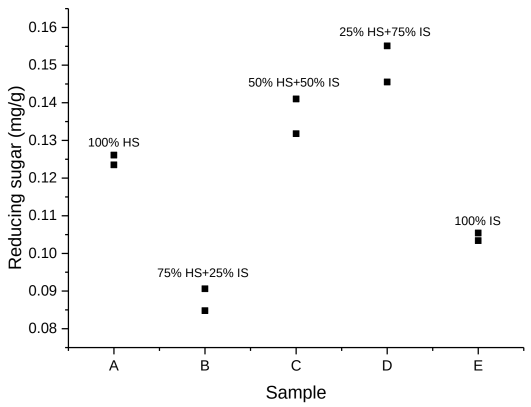

Figure 2. Reducing sugar of oil palm stem with five mixtures

Spectrum of Oil Palm Trunk

Figure 3 showed the original spectrum of oil palm trunk and 5 different mixtures produce different reflectance at any measured spectrum (200-800nm). This trend could be a basis to classify the mixture of health and infected trunk using spectrum data. It was informed that the original reflectance spectrum of oil palm trunk (Figure 3) did not show any valley or peak that can be used for selective spectrum analysis. Therefore, in this study, only full spectrum analysis was performed to develop the prediction model. As can be seen on the original spectrum, DRS UV/Vis produces high noise at every wavelength measurement. Unlike specular reflection that reflects all light at certain angle, DRS UV/Vis reflects a scattered light (Francis, 1998) resulting high noise of spectrum. However, DRS UV/Vis was utilized since it commonly used and could collect spectral information on powdered sample (Francis, 1998; Flint, 1982). In diffuse reflectance, some light travels deeper into medium and another returns toward the surface which bring spectral information of sample (Myrick, 2011). Here, in order to reduce the noise of spectrum, several preprocessing were utilized.

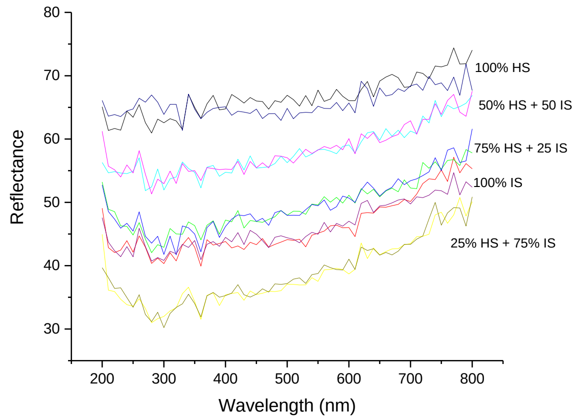

Figure 3. Original spectrum of oil palm stem

Qualitative and Quantitative Analysis

Principal Component Analysis (PCA) was performed to classify 5 mixture of sample. PCA will redure high dimension of spectral dataset into new informative variable (principal component/PC) that represent most of the data (Beattie, 2021). Using spectral data, PCA (Figure 4) could well classify 5 different mixture of sample of 75% HS + 25% IS, 50% HS + 50% IS, 25% HS + 75% IS and 100% IS. However, only 100% HS sample could not be classified. Small number of spectral data might be considered to lower the performance of PCA.

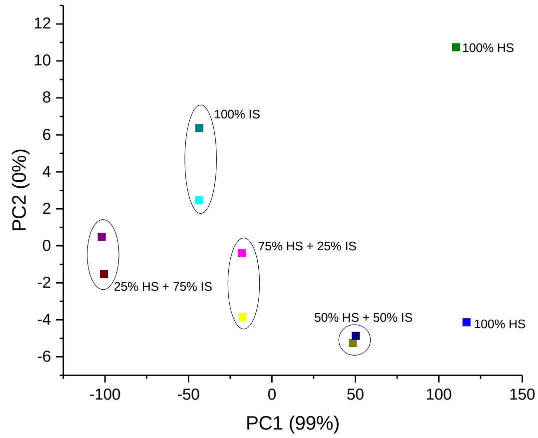

Figure 4. Score plot of oil palm stem spectrum

In order to quantify BSR based on reducing sugar, PLSR was utilized to develop prediction model. Spectrum data was set as predictor while reducing sugar as response. During PLSR calculation cross validation was performed since it better use for small sampling data rather than test matrix validation. As seen on Table 1, PLSR did not successful to do quantification of reduction sugar. PLSR is a linear modelling regression which applied for linear combination of data (Helland, 2001). From the previous discussion of Figure 2, reducing sugar did not follow a linear pattern. Even several preprocessing data had been applied, the best model that can be developed, only produce low R^2 cal (0.218) and R^2 val (0.093) using normalization (Figure 5a); so, the analysis was continued using SVR. From Table 1, SVR showed a promising result. SVR is machine learning algorithm that can handle nonlinear data (Chanda, 2019). From the result, there was an improvement for the coefficient of determination. Without any preprocessing data, SVR provide R^2 cal and R^2 val accounting for 0.744 and 4.3×10^{-8} respectively. When preprocessing data was applied to the spectrum data, the performance experience an improvement where DG1 provide the best model with high R^2 cal (0.823) and R^2 val (0.701) and low RMSEC (0.012) and RMSECV (0.028).

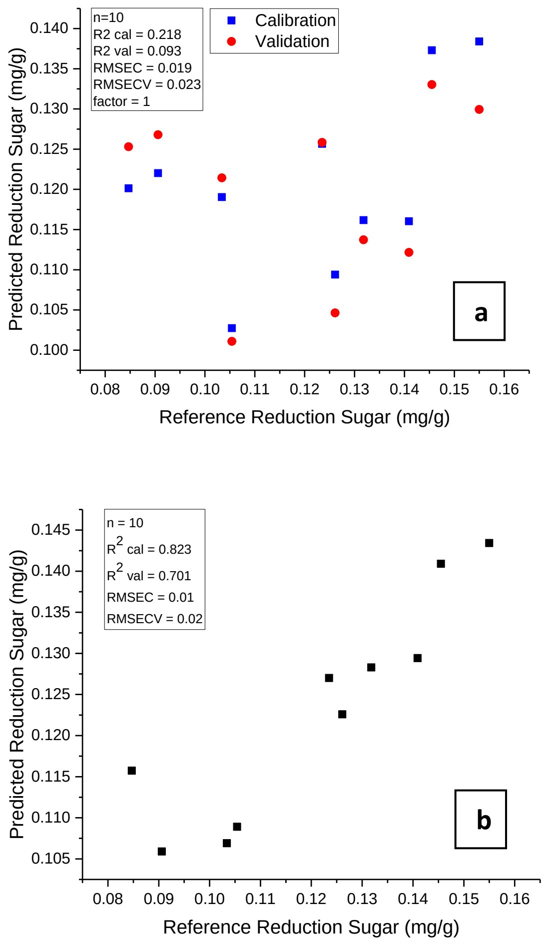

Figure 5. (a) PLS model using normalization preprocessing and (b) SVM model using DG1 preprocessing

Table 1. Statistic description of prediction model

Analysis	Pre-processing	R^2 Cal	R^2 Val	RMSEC	RMSECV
PLS	Non	0.050	NA	0.022	0.027
	MSC	0.180	NA	0.020	0.025
	SNV	0.246	NA	0.019	0.025
	DG1	0.692	NA	0.037	0.141
	Normalize	**0.218**	**0.093**	**0.019**	**0.023**
SVR	Non	0.744	$4.3x10^{-8}$	0.016	0.024
	MSC	0.619	0.425	0.016	0.0274
	SNV	0.564	0.404	0.016	0.027
	DG1	**0.823**	**0.701**	**0.012**	**0.028**
	Normalize	0.715	0.276	0.013	0.0277

CONCLUSION

Qualitative and quantitative analysis of BSR was carried out on oil palm trunk sample based on reducing sugar. It had been found that varying different mixture of health and infected trunk (to simplify different stage of Ganoderma boninese infection) could not represent an actual condition of BSR. future research should rely on actual BSR infection stages and optimum application of UV/Vis DRS combined with chemometrics need to be discovered. Regardless, PCA could classify 5 mixtures based on its spectral data, which then gave an opportunity to be applied for classifying infection stage of BSR.

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