Discriminant Analysis with Visible Lighting Properties for White Root Disease Infected Rubber Tree

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ABSTRACT
The most serious disease known in rubber industry is root disease and among the major root diseases, white root is the most destructive agent of trees and agricultural crops. Since, it is too difficult and expensive to treat root disease infection on trees; prevention is important whereby one must rely on symptoms appearing on roots themselves in order to recognize the disease. Infection symptom on the tree is detected when leaves became discolored yellowish and dying. The main objective of this work is to investigate empirically an infected rubber trees infected by white root disease where its symptom could be detected visually from leaves gradual discolouration. Visible spectrum of optical measurements is taken on four different regions of interest (ROI) locations of the top side leaf sample features such as petiolule, main vein/midrib, vein and leaf cell of rubber trees. Statistical techniques is used to analyse for conclusive scientific findings of which ROIs above has shown clear discrimination between the healthy, medium and worst condition. The scope of work involves raw data inclusion of leaf samples belong only to 2025 rubber tree clone. This clone is recommended by Rubber Research Institute of Malaysia (RRIM) management due to its popularity and commercially used by small scale planters. Outcomes of this work has suggested that only main vein/midrib and leaf cell ROIs produced convincing significant discrimination between healthy, medium and worst case. Thus, their measurements can be recommended for developing on the shelf technology engineering sensor instrument using non-invasive advanced signal processing techniques and intelligent system for early detection of white root disease.

Keywords: rubber tree, white root disease

1. Introduction
Rubber is one of the most important products that have improved Malaysian economy for the past decades [1]. Every year, large amount of latex from rubber trees are being produced and due to the increasing of the rubber products in the market, it is a necessity to maintain the quality and quantity of this latex. Therefore, health of the rubber trees must be taken seriously so that they are free from any diseases. Not only that, issues pertaining yield should be a significant topic in the upstream production. This would lead to proper management on crop improvement and protection in producing new rubber clones that not only would improve latex and timber yield, but also have vigorous growth, good tree morphology, and resistant to wind, diseases and brown-bast [2]. It is known in this industry
that diseases of rubber are divided into four categories; root, panel, stem and branch and leaf diseases [3]. Root disease is the most serious of all diseases in rubber plantation because the fungi kill the tree slowly [4].

Not only that, this disease could spread from tree to tree by root contact. Among the major root diseases, white root is a well-known and most destructive agent of trees and agricultural crops especially hevea brasiliensis. It was also reported to aggressively kill several other agriculture crops and fruit trees [4]. It is too difficult and expensive to treat trees that have been infected by root disease. So, prevention is important compared to treatment because of the constraint of money and time [5]. One must rely on symptoms appearing on the roots themselves in order to recognize the disease. Other symptom on the tree is when leaves go through discolouration and after they turn yellow, the tree will die. Conventionally, early detection of diseases is carried out via visual inspection by an expert person regularly where sometimes decision is made after matching the above infected features with the closest appearance photos from a library text. These evaluation processes however, are time consuming, have low percentage accuracy, as well as costly [4]. From the published work described in these literatures [6-8] digital imaging techniques and optical measurements could be employed to examine the leaves of infected rubber trees.

Normally, infected rubber trees would show symptoms of leaf discoloration and a previous study on empirical RGB (Red Green Blue) color extraction of these leaves through captured digital images has shown that various rubber tree diseases can be discriminated from one another [9]. Further analysis was also done on reflectance light properties using spectrometer [7]. The statistical results obtained show that there is strong evidence that these diseases can be discriminated. However, detection on color variegation and variation of the leaves at this stage is considered to be quite late for any remedial action [5]. Therefore, it is a novel idea to develop on the shelf technology engineering sensor using advanced signal processing techniques and intelligent system for non-invasive and early detection of white root disease. Such development needs fundamental studies for significant and appropriate feature extractions from the pathological perspectives of leaf discoloration [2].

2. Methodology
2.1. Introduction
This section discusses the data collection processes, the instruments used and the measurement procedures in this research. This is followed by the descriptions of the experimental set-up for quantification of the data images and elaboration of the proposed statistical methods for discriminations. The spectrum responses after the respective ROIs’ were being measured with respect to healthy, medium and worst case would also be shown. Before the analysis of optical measurements can be done, it is important to discuss the methodology which basically involves the data collection steps, type of instrument used and measuring procedures. White root disease is selected as this infection is generally common for all rubber trees regardless of which clone they came from. The next step would be the collection of rubber tree leaf samples which at a later stage were divided into three groups, categorized as healthy, medium and worst case with respect to infected white root disease. These leaves were then being measured using optical instrumentation for their reflectance quantifications. Prior to that, this instrument was calibrated in order to give a controlled specification about the surrounding condition so that any parameters being measured inevitably would produce good and reliable measurements. Finally, these measured data were numerically analysed using statistical tool to highlight significant findings and conclusion.

2.2 Instruments
MCS 600 series is the latest MicroSpectrosCopy innovated with the finest photodiode array technology introduced by Carl Zeiss [11]. With the process-tested spectrometers, it performs even the most challenging measurements in UV (Ultraviolet), VIS (Visible), NIR (Near-Infrared) spectral regions quickly, efficiently and precisely. The cost-effective MCS 600 systems as shown in Figure 1, featuring the time-tested, modular principle of the MCS 500 series with a number of major enhancements.
Highlights of the new design include an increased signal-to-noise ratio, improved wavelength accuracy and an intelligent housing system, allowing different UV/VIS/NIR spectrometers to be directly combined for the very first time – covering the spectral range from 190 to 2150 nm. In addition, the MCS 600 sets a new standard for data processing through robust communications with peripheral systems and databases employing protocols [11]. For space, application or energy reasons, some specific applications require reflectance measuring heads with an integration sphere, the maximum diameter of which measures 30 mm. The model OFK 30 measuring head shown in Figure 2, has therefore been added to the MCS 600 accessory program [23]. It has the following specifications: Meas. geometry: d/8°; Sphere diam.: 30 mm; Range: 220 – 2200 nm and Illumination: ext. light guide, d=4 mm.

For the purpose of this research, some modification was being made to the OFK head where a small hard cardboard of the same surface area size at its bottom was pasted to it. In the cardboard, a small rectangular window of 1cm in length and 3mm in width was being cut right at the position of the lens window of the head as depicted in Figure 3. The background color of the cardboard is black so that it could absorb any reflected white light during the measurement process. In this case, the transmitted and reflected light energy generated from the MCS 600 would only travel through the available rectangular window. The small window size is enough and suitable to be placed at the respective region of interest (ROI) locations of each leaf sample.
2.3. Software: Aspect Plus
Aspect Plus is a flexible, modular spectral analysis software package that runs under MS Windows and is the universal spectroscopy program from Carl Zeiss. Aspect Plus guarantees extensive functions as pictured in Figure 4, combined with outstanding ease of use. The File menu contains operations that are used for managing files that have been created using Aspect Plus or other compatible programs. Aspect Plus supports the import/export of GRAMS (spc) ASCII (csv) and JCAMP format files. The Edit menu contains operations that are useful for inter-program transfer of data. A Copy command allows a spectrum to be easily inserted into Windows-based programs such as word processors. The View menu contains operations that can be used to alter the visual display of spectra and the set-up of the screen.

Figure 3. Hard cardboard (1cm x 3mm) rectangular window attached to OFK head

Figure 4. Aspect Plus display features
2.4. Calibration
Calibration is a mandatory procedure for any measuring equipment particularly spectrometer [15]. Before proceeding with any new measurements, calibration of the OFK head must be done depending on the spectrum usage during measurement i.e. whether for the VIS and NIR spectrum. Description about calibration in this section is on VIS spectrum where dark reference has to be measured firstly by putting the OFK head on a dark or black material as demonstrated in Figure 5. After that, the measure button was activated producing a reference spectrum representing dark color. As expected, all recorded percentage reflectance for the VIS spectrum are confined to 0% as depicted in the figure. The result indicates that all light components were not being reflected from the material. These data were then saved in the system database.

![Figure 5. OFK head calibration with dark reference](image)

The whole procedure was then being repeated for measuring white reference by replacing the black material with white material as shown also in Figure 6. However this time as observed in the figure, all recorded percentage reflectance are confined to 100%, suggesting that all light components were reflected from the material. Again, these data were also saved in the system database. The final stage of calibration process was on testing the OFK head onwards a green based color material as shown in Figure 7. It is observed that the peak recorded percentage reflectance is between 510-550nm, which is actually the range for green component. Finally, these measured data were also being saved.
Figure 6. OFK head calibration white reference

Figure 7. OFK head calibration green reference
2.5. Data Collection
Samples of rubber tree leaf were collected from nursery fields at the Research Station, Rubber Research Institute of Malaysia (RRIM), Sungai Buloh. Plucking of the leaves was done by senior workers from the Crop Improvement and Protection Unit (CIPU) as soon as the infected trees with white root disease were first identified by their research officers. There are two conditions of infected trees; medium and worst conditions which are reflected by the degree of leaf discolouration while the other condition is the healthy ones. Figure 8 shows clearly examples of leaf green discolouration or degrading for the healthy, medium and worst conditions respectively.

![Healthy](image1)
![Medium](image2)
![Worst](image3)

**Figure 8.** Leaf samples of rubber tree in (b) and (c) are infected by white root disease

2.6. Measuring Procedure
Optical measurements were taken on four different regions of interest (ROI) locations of the top side leaf sample. The four locations are the petiolule, midrib or main vein/midrib, vein and the leaf cell as shown in Figure 9(a)-(d).

![Optical measurement locations on leaf](image4)
![OFK head positioning on petiolule](image5)
![OFK head positioning on main vein/midrib](image6)
![OFK head positioning on vein/cell](image7)

**Figure 9.** MCS600 Spectrometer & OFK head measurements on selected ROI locations
The top side was preferred compared to the underside of the leaf in order to be consistent with other research works on investigating leaf color spectrum [7,9]. A total of 70 leaf samples of 2025 rubber tree clone were measured for each respective ROI’s VIS spectrum reflectance reading that would represent three cases; healthy, medium and worst case. The numbers are enough for producing frequency plot that follow at least a normal distribution [12]. Healthy tree is the one not being infected by any diseases while medium case is where the tree is being infected by white root disease but categorized as early stage of infection. Finally, the worst case belongs to trees where their roots are badly infected by the white root disease and need to be treated urgently [10].

3. Results

3.1. Normality Test

The outcomes of the normality tests applied to all the data sets, are then being recorded and analysed. Measurement outcomes by the Kolmogorov-Smirnov (K-S) tests have produced significant p-values in the range from a minimum of 0.061 to a maximum of 2.00. Since these values are greater than 0.05, there is evidence that each ROI data set follow the null hypothesis of normal distribution. The summarized K-S test result for all data set is tabulated in Table 1. From the table, only healthy ROI group belongs to vein and leaf cell shows very minimal p-value of slightly equal to 0.05. Thus, indicating that these groups show weak evidence that their data are normally distributed. Such interpretations are consistent with the visual analysis made upon the graphical plots in the previous section. Overall, all of these measurements conclusively suggest that the 70 data sets can be used in the next experimental stage of inference between population either through parametric graphical methods (error bar plot) or independent t-test.

| ROI               | Statistic | df (degree of freedom) | Sig. (2-tailed) (p-value) |
|-------------------|-----------|------------------------|--------------------------|
| petiolule_healthy | .068      | 70                     | .200                     |
| petiolule_medium  | .098      | 70                     | .092                     |
| petiolule_worst   | .085      | 70                     | .200                     |
| main vein/midrib_healthy | .070 | 70 | .200 |
| main vein/midrib_medium | .069 | 70 | .200 |
| main vein/midrib_worst | .074 | 70 | .200 |
| vein_healthy      | .128      | 70                     | .065                     |
| vein_medium       | .069      | 70                     | .200                     |
| vein_worst        | .050      | 70                     | .200                     |
| leafcell_healthy  | .103      | 70                     | .061                     |
| leafcell_medium   | .061      | 70                     | .200                     |
| leafcell_worst    | .095      | 70                     | .197                     |

3.2. Error Bar Plots Analysis

The error bar or interval estimator needs to be analysed after the previous normality test has being concluded. This plot provides the possible location of the group population mean (i.e. the µ) and is used whenever graphical information about the range of the predicted population mean is required from the available known samples observation of a single group [12]. In this work, error bar of all the data sets were plotted as shown in Figure 3. There are 3 types of symbols for each ROI with respect to healthy, medium and worst condition in the y-axis, representing the range of group’s population estimated of mean value. Also, each symbol has its lower confidence limit (LCL) and upper confidence limit (UCL) where the range between these limits indicates the predicted location of the
population mean, $\mu$ [13]. Measurements of the error bar plots discussed above are summarized in Table 2. The rightmost column is the range for the 95% CI between UCL and LCL while the mean values demonstrate the locations of the ROI groups as illustrated in Figure 10. From this table and with respect to the 95% Confident Interval (CI) ranges, only the main vein/midrib and leaf cell group has shown distinct for the three conditions i.e. there is difference in the mean values between healthy, medium and worst. Thus, their specifications printed in Table 2 can be used as reference for development of sensor instrumentation in the future.

![Figure 10](image.png)

**Figure 10.** Respective locations of error bars for each ROI group

| ROI           | Mean ± Std. Error | 95% Confidence Interval |
|---------------|-------------------|-------------------------|
|              |                   | LCL         | UCL         |
| petiolule_healthy | 16.19±0.30          | 15.58      | 16.80      |
| petiolule_medium   | 13.73±0.46          | 12.82      | 14.64      |
| petiolule_worst    | 16.80±0.46          | 15.88      | 17.72      |
| mainvein_healthy   | 16.02±0.19          | 15.64      | 16.41      |
| mainvein_medium    | 14.59±0.19          | 14.20      | 14.97      |
| mainvein_worst     | 20.43±0.22          | 19.99      | 20.88      |
| vein_healthy       | 10.24±0.15          | 9.93       | 10.54      |
| vein_medium        | 9.82±0.24           | 9.35       | 10.30      |
| vein_worst         | 18.34±0.15          | 18.04      | 18.64      |
| leafcell_healthy   | 6.82±0.09           | 6.64       | 7.00       |
| leafcell_medium    | 7.68±0.15           | 7.37       | 7.98       |
| leafcell_worst     | 16.85±0.17          | 16.51      | 17.20      |
### 3.3. Independent t-Test

Independent t-test is suitable for comparing means between observed samples group (conditions set in this work) extracted from two populations. The findings are based on the p-value. As elaborated in [12,14], p-value of less than 0.5 indicates there is an overwhelming evidence to infer that the two groups are significantly or can be discriminated from each other. In this experiment, all data sets were tested for equality of means with respect to each ROI group conditions. The preferred t-test outcomes are summarized and tabulated in Table 3. It can be concluded from the table that petiolule and vein groups are not recommended for optical measurement in pre-detecting of white root disease towards rubber tree clone 2025 based on leaf discolouration. The tests have proven that there is overwhelming evidence that any measured or estimated measurements for at least two conditions (in which, healthy-worst for petiolule (p-value=0.495) and healthy-medium for vein (p-value=0.115) would occupy the same range or location. Alternatively, the tests applied for main vein/midrib and leaf cell group has produced p-values less than 0.05, implying that these groups are overwhelmingly significant different between healthy, medium and worst case. Thus, t-test has numerically reinforced the findings from the error-bar plot investigation.

| ROI | (I) category | (J) category | Mean Difference (I - J) | Std. Error | Sig. (2-tailed) (p-value) | 95% Confidence Interval LCL | UCL |
|-----|--------------|--------------|------------------------|------------|--------------------------|-----------------------------|-----|
| petiolule | healthy case | medium case | 2.46* | 0.59 | 0.000 | 1.31 | 3.62 |
| | worst case | -0.40 | 0.59 | 0.495 | -1.56 | 0.75 |
| | medium case | healthy case | -2.46* | 0.59 | 0.000 | -3.62 | -1.31 |
| | worst case | -2.86* | 0.59 | 0.000 | -4.02 | -1.71 |
| | worst case | healthy case | 0.40 | 0.59 | 0.495 | -0.75 | 1.56 |
| | medium case | 2.86* | 0.59 | 0.000 | 1.71 | 4.02 |
| main vein/midrib | healthy case | medium case | 1.44* | 0.29 | 0.000 | 0.87 | 2.01 |
| | worst case | -4.41* | 0.29 | 0.000 | -4.98 | -3.84 |
| | medium case | healthy case | -1.44* | 0.29 | 0.000 | -2.01 | -0.87 |
| | worst case | -5.85* | 0.29 | 0.000 | -6.42 | -5.28 |
| | worst case | healthy case | 4.41* | 0.29 | 0.000 | 3.84 | 4.98 |
| | medium case | 5.85* | 0.29 | 0.000 | 5.28 | 6.42 |
| vein | healthy case | medium case | -8.10* | 0.26 | 0.115 | -8.61 | -7.59 |
| | worst case | -8.51* | 0.26 | 0.000 | -9.03 | -8.00 |
| | medium case | healthy case | 8.10* | 0.26 | 0.000 | 7.59 | 8.61 |
| | worst case | 8.51* | 0.26 | 0.000 | 8.00 | 9.03 |
| leaf cell | healthy case | medium case | -10.04* | 0.20 | 0.000 | -1.25 | -0.46 |
| | worst case | -10.43* | 0.20 | 0.000 | -10.43 | -9.64 |
| | medium case | healthy case | 0.86* | 0.20 | 0.000 | 0.46 | 1.25 |
| | worst case | -9.18* | 0.20 | 0.000 | -9.58 | -8.78 |
| | worst case | healthy case | 10.04* | 0.20 | 0.000 | 9.64 | 10.43 |
| | medium case | 9.18* | 0.20 | 0.000 | 8.78 | 9.58 |

* The mean difference is significant at the 0.05 level.
4. Conclusion
In this research, the instruments used were reliable and the measuring procedures were set with professional guidance from the expert domain and also from previous research work guidelines [7]. The optical measurements of the identified ROIs of rubber tree leaves were done by using the measuring head OFK 30 that was connected to MCS 600 instrument. A total of 640 measurements were collected in this work from the identified four ROIs. Each ROI has 210 reading samples with 70 each representing the healthy, medium and worst condition of the rubber tree with respect to white root disease infection. Initially, the measuring head scanned for 120 reflectance percentage samples that correspond to the visible spectrum (VIS) range from 367.88nm to 781.62nm during measurement of each ROI. However as far as this research is concerned, only reflectance reading that represent green wavelength (546nm) was considered and hence, each ROI with the respective condition has 70 recorded readings for numerical analysis.

Graphical methods such as the interval estimator or error-bar plots were then used for visual analysis of ROI groups’ discrimination. The error bar plots displayed the 95% CI range of the possible location of population mean representing each ROI group with respect to types of conditions. It was observed that only main vein/midrib and leaf cells show clear discrimination amongst the healthy, medium and worst condition. Multiple comparison using independent t-test for equality of means from two populations, was implemented in the last stage of the statistical tests for more definitive conclusion. In the test, condition samples from each ROI were compared with other. The measured p-values equal to 0.000, thus implying that there is an overwhelming evidence only the main vein/midrib and leaf cell ROI group are able to give significant discrimination between healthy, medium or worst condition. The test results has reinforced the findings through observations of the error-bar plots where other than these two ROI groups, petiolule and vein group are not suitable to be referred for developing optical measurements in pre-detecting infection of white root disease towards rubber tree clone 2025 based on leaf discolouration.

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