Growth Monitoring of Harumanis Mango Leaves (Mangifera Indica) at Vegetative Stage Using SPAD Meter and Leaf Area Meter

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Abstract. Current advancement in orchard farming has results in ultra-high density planting of harumanis mango trees under greenhouse production system. The innovative approach in monitoring the nutrient status is deemed necessary to manage the growth of the trees. Sufficient application of nutrient especially nitrogen will exhibit healthy green color on the leaf while deficiency in nitrogen will result in poor leaf color. The level of greenness in the leaf will indicate the overall healthiness of the plant. There are very limited studies that indicate the growth of harumanis leaves at discrete time interval during it developmental stage. The objective of this research is to study the growth development of harumanis leaves by using SPAD meter and area meter at vegetative stage. SPAD reading were taken from the leaves at the age of 2nd to 7th week by using a Minolta SPAD-502 meter. Leaves were sampled from 32 trees and statistical analysis was conducted to determine the relationship of age to the growth of the leaves for its area expansion and the SPAD reading. The result for SPAD reading of the leaves shows significant increase with the growing period while leaves area shows less significance different during the growing period. This study concluded that SPAD meter can be used as a reliable tool to indicate the growth of the leaves instead of the leaf area measurement.

1. Introduction

Harumanis is the iconic products from Perlis due to the aroma, texture and the sweetness. Lately, it has risen in demand and the selling price. However, very limited study that has been done for Harumanis grown under high density planting system (HDPS) in greenhouse especially on the nutrient management aspect. Nutrient management is very important to monitor each individual tree requirement of Harumanis trees planted under this advance orchard farming.

Generally, good management and monitoring of the growth development of the trees will result in good quality produce. The quality of the produce can be predicted from the growth performance shown by the trees. Various methods have been used by researcher to determine plant growth especially via plant tissue analysis. Plant tissue sampling is essential to determine nutrient requirement for plant growth. Visually, healthiness of the trees can be indicated by the greenness of the leaves.
Leaves that exhibit healthy green color will indicate the sufficiency in the nutrient availability in the soil while leaves with poor green color results from nutrient deficiency.

The growth of leaves and important physiological events of the trees greatly depends on the age. As example Ramírez et al.[7] agreed that the age of the vegetative stems is important to induce flowering. Leaf growth was characterized into several stages. The study from Ramírez et al.[8] has described the vegetative growth of ‘Keitt’ and ‘Tommy Atkins’ variety. According to study, vegetative growth will undergo several stages from resting period, bud break, bud elongation, early leaf elongation and elongation of leaf green stage before another flush take place. It is a repetitive and continuous process throughout the growing period. A typical leaf growth displays sigmoidal curve for the log, linear and stationary phase. In normal development, leaf increase in size during its active growth period. Abnormal growth pattern indicates the state of health of the tree.

Leaf area measurement is vital in the plant and nutritional study. Ghoreishi et al.[1] has use Li-3100 area meter to measure leaf area. In the study, simple model was established for leaf area prediction of mango and significant result was obtained for leaf growth estimation. Mokhtarpour et al.[4] claimed that some method to measure plant leaf area is time consuming. The leaf area measurement using area meter was expected to overcome the constraints of other measuring tools. In the study, leaf growth model for maize were established and significant relationships were obtained between leaf area and the other growth parameter.

On the other hand, SPAD-502 has been recognized as one of the reliable tools to determine plant nutrient status[12,13]. It provides data in numerical values that indicated the level of greenness in the leaf. Theoretically, the level of greenness is directly proportional to the amount of the chlorophyll present in the leaves. It is also directly correlated to their nitrogen concentration. Difference in SPAD reading may cause by different chlorophyll concentration present in the leaves. Higher SPAD reading will indicate healthier plants with sufficient nutrient. Netto et al.[6] claimed that SPAD meter has ability to detect nitrogen deficiency, thus it can be useful to improve nitrogen management. However, the values may vary depends to the varieties, cultivar and different development stage[10]. In Malaysia, the correlation between SPAD meter and leaf nitrogen concentration has been widely studied in paddy.

The aim of this research was to study the vegetative growth of Harumanis leaves by comparing two measuring tools using leaf area meter and SPAD meter. Correlation analysis between the SPAD reading and leaves area was carried out to determine if there is correlation between both parameters. Then, the best tools indicating leaves growth was selected based on the healthiness exhibit by the leaves.

2. Material and method

2.1. Study area

The study was carried out in a greenhouse (GH03) located at the Institute of Sustainable Agriculture (INSAT), Universiti Malaysia Perlis (UniMAP), Padang Besar, Perlis. The GPS position is N6.654321, E100.265557, at 53m above mean sea level. INSAT were located at the northern area, zone 1 of Malaysia characterized with drought in January to March followed by rainy season in September to December with maximum temperature recorded up to 40°C. The soils generally are loamy clay of lateritic in origin. The planting follows the standard plant husbandry practice for high density planting except for its pruning and micro-climate controlled. The age of the trees is one year and the trees size is almost similar due to uniform pruning. The planted areas are supplied with drip irrigation system.
2.2. Plant samples and leaf measurement
A total of 32 trees per greenhouse were selected based on completely randomized design of staggered grid sampling. Leaves were selected from a healthy stem at four different sides of the trees. Each week, five trees of the same leaves age were chosen for data collection. The age of the leaves was monitored from the date of bud emergence. For non-destructive measurement of leaves greenness, Minolta SPAD-502 meter were used. The reading was recorded in SPAD unit. Measurement using SPAD meter was taken at four point per leaf at about halfway from the leaf tip and collar; about middle point between the leaf midrib and margin. The measurement within one tree was grouped as a single sample. Then, leaves from each branch were collected for destructive analysis. The collected fresh leaves were transported to laboratory in insulated box and washed with distilled water to remove dirt. The samples were measured on the area basis by using area meter (Li-3100C, Li-Cor, Inc., Lincoln, Nebraska, USA).

2.3. Data analysis
The statistical analysis was carried out using SPSS Statistics software. In the analysis, the data collected for each tree of the same age was grouped together as a single sample. T-test paired two samples per means for p-value<0.05 were conducted to distinguish significance different between the age of the leaves for both the SPAD value and area of the leaves. The relationship of the SPAD value and the area to the age were evaluated and lastly, correlation between the age, SPAD reading and area were established using correlation coefficient analysis.

3. Result and discussion

3.1. Relationship between area expansion and the age of the leaves
The result for the area expansion affected by the age of the leaves was shown in Figure 1. The graph showed that the growth of the leaves increase with increasing leaves age. Overall growth trend shows sigmoidal curve (double curve). These observations are in line with the result obtained by Gonzalez et al.[2] for leaves growth. From the graph, the early growth of young and in matured leaves showed a slower growth compared to the expansion period. The leaves grow actively during expansion period. During early growth from the age of 2nd to 3rd week, the growth mainly focused on the quantitative increase in cell number and less on elongation of cell. During the expansion period from the 3rd to 6th week, the leaves exhibit rapid growth in the leaves area size. At this state there are rapid increase in cell division and cell size. There was no significant different from the age of 4th to 5th weeks. In this period, the leaves may rest to store enough nutrients to promote further elongation and expansion of the leaves. The matured leaves at the age of 6th to 7th week do not show any significant changes in leaf area due to deceleration in the cell division and elongation. The growth is almost absence. In this study, more than 80% correlation was obtained between leaves area and leaves age. Thus, it is proved that the leaves area increased in parallel with the increasing leaves age.
3.2. Relationship between SPAD reading and the age of the leaves

The result for the SPAD reading related to the age of the leaves was shown in Figure 2. The graph showed that the SPAD reading of the leaves increase with increasing leaves age. From the graph, there was significant increase in SPAD reading at age 2\textsuperscript{nd} week (average 17.3 SPAD units) until reach peak at 6\textsuperscript{th} week (average 40.36 SPAD units). However, the value of SPAD reading dropped at 7\textsuperscript{th} week to average of 34.34 SPAD units. The greenness of leaves measured by using SPAD meter was positively correlated with chlorophyll content in the leaves. Thus, increase in SPAD reading results from increase in chlorophyll content. Decrease in SPAD reading during 7\textsuperscript{th} week in matured leaves may relate to several factors that may disturb the SPAD reading prior to measurement. When too high chlorophyll concentration exists in the leaves, it could disturb the accuracy of the instruments[9]. On the other hand, the light intensity during the time of measurement may give influence to the measurement of the SPAD reading as agreed by Martínez and Guiamet[3]. According to Nauš et al.[5], light will affect the chlorophyll distribution in the leaves due to the chloroplast movement. The non-uniform chlorophyll distribution in the leaves also may disturb the reading of SPAD meter[11]. Technically, SPAD meter needs to be controlled by user to eliminate error in the reading during the field measurement.
3.3. Correlation between area and SPAD reading
The correlation between SPAD reading and area of the leaves were shown in Figure 3. The graph shows positive gradient between both parameters. A moderate positive correlation of 62.89% was obtained between SPAD reading and leaf area measurement. Between the parameter itself, both of the leaves area and SPAD reading shows significant different since the p-value is less than 0.05. On the other hand, interaction effect between the SPAD reading and leaf area measurement do not give significant different. However, due to the higher F value in the SPAD reading compared to leaf area measurement, it was concluded that the SPAD reading able to give more accurate value indicating the healthiness of the Harumanis mango plant.

![Figure 3. Correlation between SPAD reading and area of the leaves.](image)

4. Conclusion
As conclusion, it was proved that both leaf area and SPAD measurement could give significant different to indicate plant healthiness. However, the value from SPAD meter were found to give higher significant different between the ages of the leaves compared to the leaf area measurement. Based on the result and consideration to select the best method, SPAD meter was chosen as a reliable and effective tool to aid in the evaluation of growth performance in Harumanis plants. These result consistent with the claim from previous findings that strongly support the use of SPAD meter for determining plant nutrient status. Thus, it can be elucidated that the SPAD data may be used as the ground truth for remote sensing of mango farm. This study expected to act as benchmark to indicate healthiness of Harumanis leaves at specific development period. However, other important information such as chlorophyll and total nitrogen content is still lacking. Therefore, further study is required to fill the gap in Harumanis mango farming.

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