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RESEARCH ARTICLE

Chlorophyll dynamics of field-planted Harumanis Mango (*Mangifera indica* L.) at flowering and fruiting stages using SPAD-502 measurements

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ABSTRACT

Chlorophyll plays a fundamental role in photosynthesis and contributes significantly to plant growth and yield. However, limited information is available on chlorophyll dynamics in tropical fruit crops, particularly mango. This study evaluated chlorophyll dynamics of field-planted Harumanis mango at two different growth stages (flowering and fruiting). The chlorophyll status of Harumanis mango leaves was measured using the Soil Plant Analysis Development (SPAD-502) chlorophyll meter. The SPAD chlorophyll index of Harumanis mango trees at both stages was described and compared using statistical analysis. During the flowering stage, the SPAD chlorophyll index ranged from 43.10 to 124.00 with a mean of 67.59. Meanwhile, during the fruiting stage, SPAD values ranged from 35.60 to 95.60, with a mean of 62.46. The variability in SPAD index could be related to dynamic changes in chlorophyll allocation during both growth stages. The frequency distributions of SPAD chlorophyll index analysis indicated that SPAD values did not follow a normal distribution, possibly due to physiological differences between the two stages. The SPAD chlorophyll index differed significantly across flowering intensity stages but not across crop load stages. A decreasing trend in chlorophyll content was observed with increasing flowering intensity and crop load, suggesting possible effects of nutrient resources and availability. Overall, this study enhances the understanding of chlorophyll dynamics in Harumanis mango and offers insights for improving sustainable orchard management of this economically important tropical fruit.

1. INTRODUCTION

Chlorophyll is the main pigment responsible for the green colour of plants and other photosynthetic organisms. In plants, chlorophyll content may reflect photosynthetic ability as well as the overall plant's health (Croft et al., 2017; Pavlovic et al., 2015; Yu et al., 2024). During photosynthesis, chlorophyll absorbs light energy and transforms it into chemical energy. Chlorophyll can be found in the chloroplasts of plant cells, and it is essential for photosynthesis, the process by which plants convert sunlight into energy and produce their own food. Because it accepts light absorbed by other pigments, chlorophyll is referred to as the primary pigment of the photosynthetic reaction (Tantray et al., 2020). Chlorophyll also plays a crucial role in determining crop performance, particularly through its association with nutrient status (Lee et al., 2019; Liu et al., 2019). The colour of plant leaves, which is related to the amount and percentage of chlorophyll, can indicate nutrient status. Thus, leaf colour may serve as an index of plant nutrient condition. The strong relationship among chlorophyll content, plant health, and nutrient availability underscores its importance to overall plant productivity. Chlorophyll content is also closely linked to nitrogen levels and can influence the final yield of many crops (Carranca et al., 2018; Liu et al., 2019; Sid'Ko et al., 2017).

Nitrogen plays an important role in the production of chlorophyll, which is responsible for photosynthetic processes in plants. Studies have revealed that increased nitrogen application can raise chlorophyll content in many fruit trees, such as apple (Lee et al., 2019; Uysal, 2018; Yakovenko et al., 2021), apricot (Khasawneh et al., 2021), pear (Sete et al., 2019), mandarin (Stander et al., 2017), and papaya (Cavalcante et al., 2016), which in turn raises plant photosynthetic rates (Wang et al., 2021).

A widely applied technique for monitoring plant physiological status is measuring chlorophyll concentration.

Besides assessing chlorophyll, such measurements can also provide information about nitrogen uptake, maximum fluorescence, carotenoids, and other physiological traits. Normally, chemical extraction of chlorophyll from plant specimens is the standard procedure for measuring chlorophyll concentration (Kalaji et al., 2017; Kamble et al., 2015). This involves mechanically dissociating and dissolving the leaves with chemicals (e.g., acetone), filtering the chlorophyll from the other plant compounds, and then measuring the chlorophyll concentration with a spectrophotometer (Ašimović et al., 2016). Since chemical extraction is a destructive, labour-intensive, and time-consuming method for measuring chlorophyll directly, mobile optical scanners have been developed as a non-destructive, but relatively expensive, alternative for assessing plant chlorophyll levels. Over the past three decades, several non-destructive approaches for assessing plant chlorophyll content have been developed to improve the research and development on crop physiology (Ali et al., 2021; Pérez-Patricio et al., 2018). Non-destructive chlorophyll estimation techniques have been successfully employed to monitor and manage agricultural fields, including forested areas, deforestation processes, and the spread of exotic species.

One of the most widely used non-destructive devices is the SPAD-502 chlorophyll meter (Konica Minolta, Japan), which estimates chlorophyll concentration in leaves based on light absorbance at specific wavelengths (Dadhich et al., 2023; Zhang et al., 2022). The SPAD-502, known as a Soil-Plant Analysis Development chlorophyll meter, is also a handheld device commonly used as a proxy for leaf nitrogen concentration. It is often used in agricultural and horticultural research to assess plant health and nutritional status. The SPAD value, typically ranging from 0 to 100, is a common measurement derived from this process, where higher values indicate greater chlorophyll content and often healthier plants. Researchers and farmers use SPAD chlorophyll meters to inform decisions on fertilisation, crop management, yield prediction, and overall plant health (Talebzadeh and Valeo, 2022; Zhang et al., 2022). However, despite its widespread application in temperate fruit crops, information on SPAD-502 chlorophyll dynamics in tropical fruit trees, particularly Harumanis mango, remains limited.

Nonetheless, very few studies have been conducted on the chlorophyll pattern of tropical fruits, especially mango. Additionally, understanding the effects of the growing environment on the physiological mechanisms in fruit trees is essential to improve sustainable crop management practices in tropical mango. Therefore, we conducted this study to evaluate chlorophyll status in the tropical mango (Mangifera indica L.) cultivar Harumanis. A few studies conducted on many fruit crops have shown that chlorophyll status can be influenced by various factors such as environmental signals (e.g., light, temperature, and endogenous hormones) (Lu et al., 2025), cultural practices (e.g., pruning and rootstock) (Dayal et al., 2016), nitrogen application (Khasawneh et al., 2021; Mao et al., 2024; Uysal, 2018), and growing conditions (Ahmad et al., 2023; Roslan et al., 2019). However, the Harumanis mango cultivar is a relatively new fruit crop that requires greater attention in research and development. Most recent studies focus only on Harumanis mangos planted under rain protection structures or greenhouses (Helmey et al., 2023; Jaafar, 2017; Talib et al., 2020), not those planted in the open field.

Although Ahmad et al. (2023) previously investigated the relationship between the SPAD index and total chlorophyll content in Harumanis mango grown in a greenhouse, their study was limited to developing a regression model and spatial mapping of chlorophyll distribution within a controlled environment (i.e., greenhouse planting). Such controlled settings minimise environmental variability but do not capture the complex interactions between physiological and environmental factors that affect chlorophyll dynamics in open-field conditions. In addition, existing studies on Harumanis mango primarily emphasise growth performance (e.g., Helmey et al., 2023) or spatial chlorophyll mapping (Roslan et al., 2019) under protected environments, which do not fully capture the complex interactions between crop physiology and fluctuating field conditions.

Therefore, the present study was conducted to evaluate chlorophyll dynamics in field-planted Harumanis mango trees under natural climatic conditions. Specifically, this study aimed to characterise variations in the SPAD chlorophyll index during two critical reproductive stages, flowering and fruiting, and to examine the effects of flowering intensity and crop load on chlorophyll status. By monitoring chlorophyll patterns from controlled greenhouse environments to actual orchard conditions, this study advances the physiological understanding of Harumanis mango and helps address issues related to sustainable production and environmental conditions for this high-value fruit crop.

2. MATERIALS AND METHODS

2.1. Study site and planting materials

The study was conducted during the 2022-2023 growing season at an experimental Harumanis mango orchard located in Beseri, Perlis, Malaysia (6°33'03.0" N, 100°13'32.8" E), managed by a Good Agricultural Practices (GAP)- certified grower under the supervision of the Department of Agriculture (DOA), Perlis State. The area experiences a tropical climate typical of Northern Peninsular Malaysia, with seasonal rainfall and temperature fluctuations that reflect the commercial open-field conditions for

Harumanis mango production. Eight-year-old *Mangifera indica* L. cv. Harumanis trees were selected for this study. Trees were planted at a spacing of 7 m × 7 m and managed following standard local orchard practices, including routine fertilisation, irrigation, and weed control. To minimise variability unrelated to treatment effects, only trees with uniform canopy architecture, trunk diameter, and overall vigour were selected in this study. This selection ensured that observed differences in chlorophyll status were primarily associated with growth stage, flowering intensity, and crop load rather than inherent tree-to-tree variation.

2.2. SPAD-502 chlorophyll meter measurements

The leaf chlorophyll status of Harumanis mango leaves was measured non-destructively using a SPAD-502 chlorophyll meter (Konica Minolta, Japan) across flowering and fruiting stages (Figure 1). These stages represent periods of contrasting physiological demand and were therefore considered suitable for evaluating chlorophyll dynamics in field-grown mango trees. Thirty Harumanis mango trees (n = 30) with varying flowering intensity and crop load were selected for this study. The flowering stage was defined as the period when more than 50% of the panicles on a tree exhibited visible floral structures. In contrast, the fruiting stage was defined as the period when fruits were present and actively developing on the branches. Flowering intensity of Harumanis mango trees was visually assessed based on the approximate proportion of flowering panicles per tree and classified into three categories as follows:

- **Low flowering intensity**: < 30% of panicles were flowering.
- **Moderate flowering intensity**: 45–55% of panicles were flowering.
- **High flowering intensity**: > 70% of panicles were flowering.

During the fruiting stage, crop load was assessed by manually counting the number of fruits per tree during the fruiting stage and categorised as:

- **Low crop load**: <2 0 fruits per tree.
- **Medium crop load**: 30–50 fruits per tree.
- **High crop load**: > 70 fruits per tree.

These classifications reflect typical orchard-based assessment practices and were adopted to facilitate consistent comparison of chlorophyll dynamics under varying reproductive demands.

For each tree, five to six fully expanded, healthy leaves were selected from the mid-canopy region and exposed to similar light conditions. Measurements were taken on the leaf lamina while avoiding major veins and the midrib. All SPAD readings were conducted between 0900 and 1100 h to minimise diurnal variation in chlorophyll readings. The SPAD meter was zero-calibrated prior to each measurement session to ensure consistency. A total of 150 SPAD readings were obtained during the flowering stage (*n*=150) and 160 readings during the fruiting stage (*n*=160). The measurement method applied in this study followed the approach described by Roslan et al. (2019) and Ahmad et al. (2023) for similar mango cultivars.

2.3. Experimental design and statistical analysis

The experimental design for this study was a Completely Randomised Design (CRD) with staggered grid sampling based on flowering intensity and crop load (i.e., number of fruits). Data were compiled in a spreadsheet and summarised using a pivot table in Microsoft Excel. The SAS® software system was used to analyse and assess data normality. First, the chlorophyll index data measured with a SPAD-502 meter were described and evaluated using descriptive statistics. Second, the data were subjected to statistical analysis to assess differences between the studied groups. The SPAD chlorophyll index of Harumanis mango leaves under different flowering and fruiting conditions was analysed using One-Way ANOVA followed by Duncan Multiple Range Test at $P \leq 0.05$. In addition, contrast analysis was used to compare two different stages (i.e., flowering and fruiting). Lastly, the data were analysed using regression to evaluate the relationships among the variables in this study.

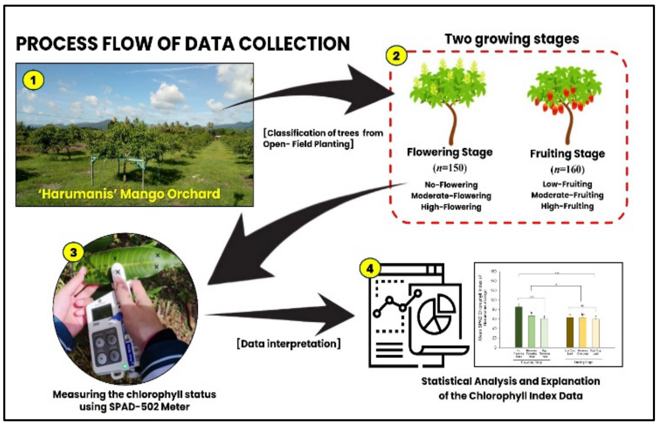

Figure 1: Process flow diagram of chlorophyll status measurement of Harumanis mango leaves using a SPAD-502 Chlorophyll Meter (Konica-Minolta, Japan) at two different growth stages (i.e., flowering and fruiting). The classification is based on flowering intensity and crop load, followed by statistical analysis of chlorophyll index data.

3. RESULT AND DISCUSSION

3.1 Descriptive analysis of SPAD Chlorophyll Index of Harumanis mango

Table 1 shows the descriptive statistics of SPAD chlorophyll index measurements for Harumanis mango during the flowering and fruiting stages. The mean SPAD index during the flowering stage was 67.59, slightly higher than the mean of 62.46 recorded during the fruiting stage. This result indicates that the SPAD chlorophyll index of Harumanis mango decreased by approximately 8% during the flowering stage. In the 'Keitt' mango cultivar planted in open-field planting, a recent study reported that the average SPAD chlorophyll index was 38.17 and 41.06 in two consecutive growing seasons, and that the SPAD index can be influenced by the application of bio-stimulants (Almutairi et al., 2023).

A higher SPAD reading index found in our study (Table 1) may also indicate greater chlorophyll content in field-planted Harumanis mango trees. Observations by Kleiman and Koptur (2023) found that higher SPAD readings may have resulted from proper orchard management (i.e., free-weed management practices). Our results also showed that the range of the SPAD chlorophyll index for Harumanis mango was 43.10-124.00 during the flowering stage and 35.60-95.60 during the fruiting stage (Table 1). Meanwhile, Jamil et al. (2015) found that the SPAD chlorophyll index of subtropical mango cultivars planted in the Pakistani climate ranged from 22.47 to 47.17.

Table 1: Summary of descriptive statistics for SPAD chlorophyll index of field-planting of Harumanis mango trees at two different growth stages (i.e., flowering and fruiting stage).

Descriptive Statistics	SPAD chlorophyll index	
	Flowering Stage (*n* = 150)	Fruiting Stage (*n* = 160)
Mean	67.59	62.46
Range (Min-Max)	43.10 – 124.00	35.60 – 95.60
Median	62.20	60.55
Mode	55.90	56.00
Standard Deviation (SD)	15.68	11.95
Coefficient Variation (CV, %)	23.19	19.13
Variance	245.71	142.81
Standard Error of Mean (SEM)	± 1.28	± 0.94

The SPAD chlorophyll index readings also varied between flowering and fruiting stages (Table 1). The coefficient of variation was higher during flowering (23.19%) than during fruiting (19.13%), indicating greater heterogeneity in chlorophyll content among leaves during the flowering stage. As trees move from vegetative growth (i.e., flowering stage) to reproductive development (i.e., fruiting stage), when demands for assimilates and nitrogen increase, this increased variability probably reflects increased physiological activity and varying nutrient allocation. It could also be related to the role of chlorophyll in supporting photosynthesis and to dynamic changes in resource allocation, such as nitrogen availability, as suggested by Tantray et al. (2020).

3.2 SPAD chlorophyll index distribution at different growth stages

The frequency distribution of SPAD chlorophyll index for both growth stages is presented in Figure 2. These graphs present the data concisely, showing how frequently a variable falls into each category. The highest frequency of SPAD chlorophyll index during the flowering stage was recorded between 50 and 59. However, the highest frequency of the SPAD chlorophyll index during the fruiting stage was recorded between 60 and 69. As we can see, the frequency distribution of the SPAD chlorophyll index is similar across both growing stages (Figure 2) and does not follow a normal distribution. We also found that the distributions were skewed to the left in both growing stages (as indicated by the statistical analyses, $P<0.001$ and $P=0.0003$, respectively), possibly due to physiological variability during both stages. Differences in the chlorophyll index range in our study may be associated with dynamic changes in leaf resource allocation (Tantray et al., 2020).

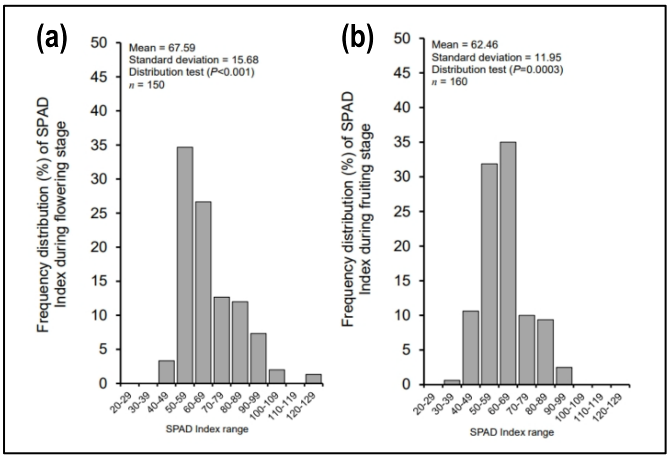

Figure 2: The frequency distribution pattern of SPAD chlorophyll index of Harumanis mango planted in the open-field conditions at two different growth stages: (a) during the flowering stage and (b) during the fruiting stage. The chlorophyll index for each stage (i.e., no-, moderate-, and high-flowering or fruiting) was pooled in the graphs.

The differences in the frequency distribution between flowering and fruiting stages may also suggest stage-specific shifts in chlorophyll distribution, likely associated with changes in photosynthetic demand and nutrient partitioning (Figure 2). During the flowering stage (Figure 2a), chlorophyll levels in leaves of Harumanis mango trees may decrease, as the plant redirects all the resources from vegetative organs (i.e., leaves) to support the formation of reproductive structures (i.e., flowers). Meanwhile, during the fruiting stage (Figure 2b), there might be a redistribution of chlorophyll to support the development of fruits. While some leaves may still be actively involved in photosynthesis, others may contribute nutrients and energy to developing fruits. Our results also indicate a wide distribution of the SPAD chlorophyll index, consistent with previous studies on other mango cultivars (Almutairi et al., 2023; Jamil et al., 2015; Kleiman and Koptur, 2023). A few studies also reported substantial variation in the SPAD chlorophyll index when plants were grown under greenhouse conditions (Ahmad et al., 2023; Roslan et al., 2019). Based on our results, the chlorophyll index measured by the SPAD meter could serve as an indicator of chlorophyll status in Harumanis mango trees across both growth stages.

3.3 Comparison of SPAD chlorophyll index at different stages

Figure 3 shows the comparison of the SPAD chlorophyll index at two different stages of the Harumanis mango (i.e., flowering and fruiting stages). There was a significant difference (*P*<0.001) in the SPAD chlorophyll index during the flowering intensity (i.e., Low, Moderate, and High) (Figure 3). A significantly higher SPAD chlorophyll index was observed in non-flowering Harumanis mango trees compared with those showing moderate and high flowering intensity. Low-flowering intensity recorded the highest mean SPAD value (85.34), followed by moderate-flowering intensity (66.73). Trees with high flowering intensity exhibited the lowest SPAD mean (60.86). It was interesting to note that high-flowering trees had a significantly lower SPAD chlorophyll index, about 30% lower than in non-flowering trees. A decline in chlorophyll levels with increasing flowering intensity may indicate greater nutrient and photosynthate allocation to floral initiation and development, thereby reducing chlorophyll retention in leaves.

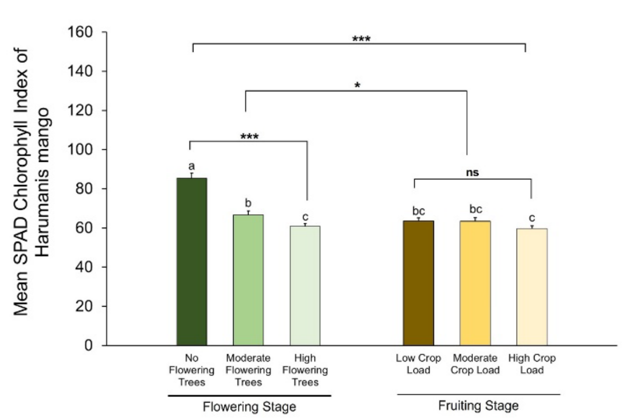

Figure 3: Comparison of SPAD chlorophyll index at different growth stages; (a) flowering stage (non-, moderate- and heavy-flowering trees) and (b) fruiting stage (low-, medium- and high-crop load) of Harumanis mango trees. Statistical differences were determined using One-Way ANOVA followed by Contrast Analysis (SAS®), with icons indicating significant means separation and *, **, *** or ns denoting levels of significance at $P \leq 0.05$. Different letters indicate significant differences between growth stages, as determined by a post hoc test using the Duncan Multiple Range Test (DMRT) at $P \leq 0.05$.

A contradictory result was found on the SPAD chlorophyll index at the fruiting stage (Figure 3). No significant differences were found on the SPAD chlorophyll index between low, medium, and high crop load Harumanis mango trees (*P*=0.20). These results could be related to the dynamics of chlorophyll content during both stages. It is hypothesised that chlorophyll content in the leaves of moderate- and high-flowering Harumanis mango trees may decrease as the plant reallocates resources to reproductive structures such as flowers and fruits. Besides that, this pattern also suggests that while reproductive sink demand during flowering strongly influences chlorophyll dynamics, fruiting intensity exerts a comparatively smaller effect, possibly due to compensatory leaf photosynthetic adjustments.

However, this would not be the sole factor, as chlorophyll content dynamics across different growth stages can also be influenced by nutrient availability, growing environment, and stressors (Li et al., 2018; Tantray et al., 2020). Our results in the present study may indicate that chlorophyll content during both growing stages is influenced by nutrient availability in the trees. A declining trend in chlorophyll level with increasing flowering intensity (Figure 3) may also explain why higher flowering intensity increases demand for nutrients, especially nitrogen, to support panicle development, thereby reducing the amount available for chlorophyll synthesis in leaves. We had speculated that major nutrient elements, such as nitrogen, may contribute to chlorophyll content in trees, as nitrogen is an essential element for chlorophyll production in plants (Carranca et al., 2018).

Previous studies reported a significant correlation between SPAD chlorophyll readings and nitrogen levels in a few temperate mango cultivars (Faria et al., 2016; Ibell et al., 2018; Rao et al., 2022). Similarly, studies on other fruit tree crops also found that the chlorophyll level was correlated with the nitrogen level in leaves, as reported in apples (Lee et al., 2019; Treder et al., 2016), grapes (Ates and Kaya, 2021), peaches (Benati et al., 2021; Rubio Ames et al., 2020), and guava (Souza et al., 2016). Therefore, the relationship between chlorophyll content and nitrogen level in Harumanis mango trees warrants further investigation.

3.4 The relationship between SPAD Chlorophyll index at different growth stages

Figure 4 examines the relationship between the SPAD chlorophyll index and different growth stages of Harumanis mango trees: (a) during the flowering stage and (b) during the fruiting stage. In general, the results showed a negative linear relationship between the SPAD chlorophyll index and flowering intensity (Figure 4a). Based on the regression equation ($y = -12.225x + 95.493$) and coefficient of determination ($R^2 = 0.92$), there was a strong relationship between SPAD chlorophyll index and flowering intensity. The SPAD chlorophyll index decreased significantly as flowering intensity increased (Figure 4a). A similar trend was observed in the relationship between the SPAD chlorophyll index and fruiting stages (low, moderate, and high crop load), as shown in Figure 4b. However, based on the equation and coefficient of determination ($y = -2.12x + 66.45y = -2.12x + 66.45y$, and

R^2 = 0.79, respectively), the relationship can be considered a moderate fit between SPAD index and crop load (Figure 4b). This result suggests that as the SPAD chlorophyll index decreases, crop load increases. Overall, we found that both flowering intensity and crop load had an inverse effect on the SPAD Chlorophyll index.

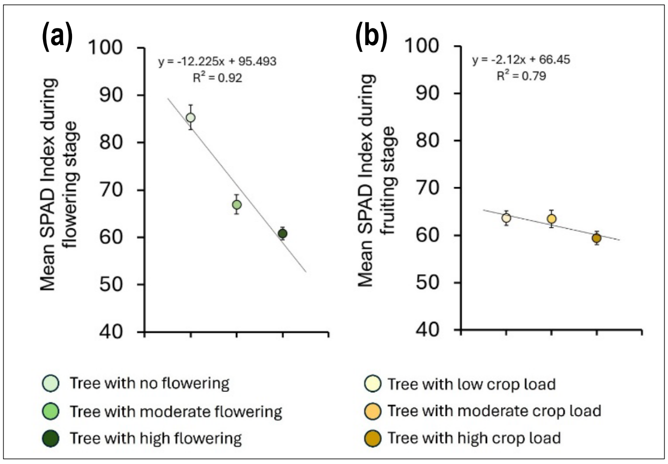

Figure 4: The relationship between SPAD chlorophyll index at different growth stages, with (a) flowering stage (non-, moderate-, and heavy-flowering trees) and (b) fruiting stage (low-, medium-, and high-crop load) of Harumanis mango trees.

Our results also showed that the steeper slope in the flowering stage of Harumanis trees (Figure 4a) indicates that flowering intensity had a more significant impact on the SPAD chlorophyll index than crop load during the fruiting stage (Figure 4b). This effect was probably due to the significant allocation of resources (i.e., possibly nutrients available) needed during the flowering process, which might reduce the tree's ability to maintain high levels of chlorophyll content in tree leaves. It was also noted that both regression lines (Figures 4a and 4b) show a negative relationship between growth stage and the SPAD chlorophyll index. This indicates that reproductive activities in both growth stages compete for resources, which might affect leaf greenness through chlorophyll concentration. Besides that, the larger effect of flowering stages on SPAD chlorophyll index may suggest a more critical period for managing nutrient supply (Faria et al., 2016; Yakovenko et al., 2021) as well as plant health status (Talebzadeh & Valeo, 2022).

Different growth stages (i.e., flowering and fruiting) can directly affect chlorophyll levels due to complex metabolic genetics (Kholmanskiy and Zaytseva, 2020) or indirectly through photosynthetic potential and leaf surface index (Yu et al., 2024), which may influence future tree productivity. It was also noted that disruptions in nutrient supply during rapid growth and fruiting inhibited physiological activities, thereby reducing chlorophyll content (Andrianto and Faizal, 2017; Croft et al., 2017).

Overall, our results suggest a strong association between chlorophyll and growth stages of Harumanis mango trees, especially during the flowering stage (Figures 3 and 4). Our findings indicate that flowering intensity has a more pronounced effect on chlorophyll content than crop load. This is consistent with the physiological understanding that flowering marks a major shift from vegetative to reproductive growth, often accompanied by significant changes in nutrient partitioning. In contrast, once fruit development progresses, trees may regulate leaf nutrient status more effectively, resulting in less variation in SPAD values across crop-load categories. As chlorophyll content is related to nutrient availability in plants (Liu et al., 2019), it would be reasonable to suggest a need for proper nutrient management for Harumanis mango to support both flowering and fruiting, as well as supporting the photosynthetic capacity of trees (Croft et al., 2017; Liu et al., 2019).

4. CONCLUSION

The findings of this study show that SPAD chlorophyll index values were generally higher and more variable during flowering than during fruiting. The broader range and higher coefficient of variation during flowering suggest greater physiological and nutritional fluctuations as the tree allocates resources toward panicle development. The resources available in the leaves of high-flowering Harumanis trees could be reallocated toward reproductive structures, such as flowers, rather than toward fruiting, thereby reducing the value of the chlorophyll index measured by the SPAD meter. Given the results above, we suggest that the chlorophyll index measured by this meter can be used to monitor the plant growth status in Harumanis mango. As noted, the relationship between chlorophyll content and plant health status is closely linked, as chlorophyll plays an important role in a plant's overall health. Besides that, chlorophyll content in plants has been widely shown to correlate with nitrogen content and plant productivity, but these aspects remain underexplored in tropical crops such as Harumanis mango. Therefore, further studies are needed to evaluate the inter-physiological relationships among leaf chlorophyll content, nitrogen level, and growing environment, focusing on the tropical Harumanis mango. A fundamental understanding of this aspect could be used to improve the orchard management of this potential crop. Based on our findings, we suggest that this method can be used by other researchers and growers to inform decisions on fertilisation, crop management, and overall plant growth status for sustainable Harumanis mango cultivation in the Northern Region of Malaysia.

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