Stomatal Conductance and Chlorophyll Fluorescence of Oil Palm under Field Conditions

MSc Thesis Plant Production Systems

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Stomatal Conductance and Chlorophyll Fluorescence of Oil Palm under Field Conditions

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Disclaimer: this thesis report is part of an education program and hence might still contain (minor) inaccuracies and errors.


Contact office.pp@wur.nl for access to data, models and scripts used for the analysis.
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Abstract

Potassium has been known to affect the stomatal movement of palms, especially during drought stress. Previous studies have observed midday closure of oil palms stomata during the dry season and some studies in other crops showed that potassium has a role in widening the stomatal aperture. In this research, we would like to know whether being potassium sufficient could widen the opening of oil palm stomata and avoid photoinhibition of photosystem II during drought stress. A pilot study was done beforehand to observe the difference of measurement result conducted at leaflets of different frond numbers and at different frond parts. Different frond numbers were recorded to have quite similar photosynthetic efficiency. Leaf senescence had influence to stomatal conductance but not to photosynthetic efficiency. Leaflets at the top layer of middle part of frond 17 were found to be the most representative leaflets for stomatal conductance and chlorophyll fluorescence measurement. In the actual experiment, palms in two different plots, including a plot with sufficient potassium (plot (+)K) and a plot with deficient potassium (plot (-)K), were set as the object of observation. Growth, chlorophyll fluorescence, and stomatal conductance parameters of palms were observed. The growth and chlorophyll fluorescence result indicated that palms at the plot (+)K, unexpectedly experienced stress indicated by lower growth and PSII efficiency. This was suspected to be caused by competition of resources from weeds surrounding the palms as a result of improper maintenance even though the fertilizer was properly applied. We observed a difference of stomatal conductance pattern during the day between palms of both plots. Palms at plot (+)K showed a significant midday closure while the palms at plot (-)K did not. The high variability of stomatal conductance in the morning period made the declining trend of stomatal conductance of palms at plot (-)K was statistically not significant. This high variability may have been caused by a small sample size that we had but as we noticed that it only happened in the morning, there was a possibility that some palms could not fully open its stomata in the morning as a result of potassium deficiency. Limited information regarding leaf potassium content and soil potassium availability limited us from exploring the other possible causes. This preliminary study has provided some useful information for further studies about the behavior of stomata under field conditions. Further research with larger sample size, more replications, and well-maintained trial, might potentially find a significant difference of stomatal behavior and photosystem efficiency between palms with different potassium treatment.
1. Introduction

1.1 Oil Palm and Water Deficiency

Oil palm (*Elaeis guineensis* Jacq.) has substantially benefited the economic well-being of smallholder farmers in Indonesia (Rist et al., 2010). However, oil palm smallholder farmers are facing problems of suboptimal yield due to a range of factors (Woittiez et al., 2017). In recent years, the threat of water stress has been becoming imminent due to increasing frequency of drought periods and climate change and there are various negative impacts on oil palm that could be caused by water deficiency (Table 1). Among those impacts, stomatal closure is particularly interesting to be studied because it could significantly reduce the rate of photosynthesis which leads to a yield reduction even if water stress has no other effects (Corley, 1981).

Table 1. Impacts of water deficiency on oil palm

<table>
<thead>
<tr>
<th>Impacts</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delays leaf opening</td>
<td>(Cros et al., 2013)</td>
</tr>
<tr>
<td>Decreases sex ratio</td>
<td></td>
</tr>
<tr>
<td>Abortion of inflorescences and infructescences (bunches)</td>
<td></td>
</tr>
<tr>
<td>Decreases photosynthesis rate due to both decrease of transpiration and impairment of photosynthetic apparatus</td>
<td>(Silva et al., 2017)</td>
</tr>
<tr>
<td>Reduces transpiration</td>
<td>(Cha-um et al., 2010b)</td>
</tr>
<tr>
<td>Reduces total dry weight and vegetative growth</td>
<td></td>
</tr>
<tr>
<td>Stimulates stomatal closure</td>
<td>(Haniff, 2006)</td>
</tr>
<tr>
<td>Reduces FFB (Fresh Fruit Bunches)</td>
<td>(Corley &amp; Mok, 1972)</td>
</tr>
<tr>
<td>Reduces leaf area</td>
<td>(Suresh et al., 2012)</td>
</tr>
<tr>
<td>Causes disturbance on photosynthetic apparatus</td>
<td></td>
</tr>
</tbody>
</table>
1.2 The Role of Potassium Role under Drought Condition

In terms of fertilization, Woittiez et al. (2015) found a yield reduction and yield gap of oil palm in smallholder farmers of Indonesia which was likely caused by insufficient fertilization, especially potassium. Potassium is a key determinant of the productivity of oil palm. It plays many roles such as increasing dry matter production and yield of oil palms, primarily by increasing leaf area (Corley & Mok, 1972). In general, potassium has an important role in crop physiological processes (Table 2). Aslam et al. (2013) reported that plants lacking K seem to develop an acute sensitivity to water deficit. Literatures suggest that potassium could alleviate the negative impacts caused by drought stress as explained below:

1. Role of potassium in osmotic adjustment during drought stress
Potassium is one of the most important osmotic in plants and the main cation in plants that are available in a soluble form which makes it closely linked to the leaf water potential (Mengel & Arneke, 1982). It acts as the main determinant of cell turgor (White, 2013) which supports osmotic adjustment and sustains cell expansion at low soil water potential (Grzebisz et al., 2013). The impact of K nutrition significantly affects many processes linked to plant water, such as leaf water content, leaf growth (measured in terms of leaf elongation rate and leaf area), and internode growth (measured in terms of plant height; Martineau et al. (2017)).

2. Role of potassium in stomatal regulation
Potassium also plays an important role in stomatal regulation (Arquero et al., 2006). It has an influence on the regulation of stomatal aperture, and hence on the limitation of water loss. Potassium is required for proper stomatal opening by providing the osmotic driving force for water influx into the guard cell vacuole (Peiter, 2011). During stomatal opening, accumulation of osmotically active solutes leads to an increase in the osmotic potential and a decrease in water potential in guard cells. A massive influx of water into the guard cell compensates this water potential decrease, leading to an increase of turgor and the swelling of guard cells. Conversely, decreasing osmolyte concentrations leads to a massive water efflux from guard cells, which results in the reduction of guard cells turgor and closure of stomata (Figure 1). Monovalent cations can affect guard cells osmotic potential, however, K⁺ is the most relevant as it accumulates at high concentrations in guard cells (MacRobbie & Lettau, 1980). K⁺ uptake is mainly responsible for the rapid increase of turgor (Talbott & Zeiger, 1996), thus, stomatal opening and closure are impaired in K limited plants (Brag, 1972).

3. Role of potassium in eliminating reactive oxygen species
Reactive oxygen species (ROS) are chemically reactive chemical species containing oxygen such as peroxides, superoxide, and hydroxyl radical. In an excess amount (for instance during stress), ROS can cause oxidative stress to plants. K has been known to lengthens leaf lifespan by elimination of ROS which are often released following a water deficit period. whereas ROS are insufficiently eliminated under K limitation (Cakmak, 2005). Such elimination maintains a photosynthetically active leaf area under stress (Christina et al., 2015).

4. Role of potassium in promoting deep root exploration and maintaining root growth
Sufficient K supply promotes root growth which contributes to higher water uptake by improving deep root exploration (Martineau et al., 2017). Furthermore, Grzebisz et al. (2013) argued that water stress is accompanied by a subsequent decrease in transpiration rate, resulting in a less mass flow of K to the root surface, which further reduces K uptake in the K-nonamended soil. Increasing K in the soil solution compensates this effect by providing an
adequate K supply and maintaining root growth which allows access to other mineral elements (including nitrogen) and water which will increase yield.

Table 2. Role of potassium in crop physiology

<table>
<thead>
<tr>
<th>Roles</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Determinant of cell turgor</td>
<td>(White, 2013)</td>
</tr>
<tr>
<td>Supporting osmotic adjustment</td>
<td>(Grzebisz et al., 2013)</td>
</tr>
<tr>
<td>Sustaining cell expansion during drought stress</td>
<td></td>
</tr>
<tr>
<td>Regulating stomatal aperture</td>
<td>(Peiter, 2011)</td>
</tr>
<tr>
<td>Support enzymes functioning i.e. starch synthase</td>
<td>(Marschner, 2011)</td>
</tr>
<tr>
<td>Promotes root growth which contributes to higher water uptake by improving deep root exploration</td>
<td>(Battie-Laclau et al., 2016)</td>
</tr>
<tr>
<td>Elimination of ROS (Reactive Oxygen Species)</td>
<td>(Cakmak, 2005)</td>
</tr>
</tbody>
</table>

Figure 1. Regulation of inorganic ion concentration in guard cells triggers the decrease of osmotic pressure and massive water efflux (Pareek et al., 2009)

Drought stress stimulates stomatal closure which is a tolerance mechanism of many plants to avoid water loss. However, stomatal closure also means a reduction in total assimilation rate and slower plant growth. There is a possible improvement in WUE if stomata respond rapidly (almost instantaneously) and in synchrony with mesophyll demands for CO₂ (Lawson & Blatt, 2014). Stomatal movements arise from the transport, accumulation, and release of osmotically active solutes and according to Humble and Hsiao (1969), potassium (in a form of K⁺ ion) is the primary inorganic solute involved in that process.
1.3 Problem Statements

Rees (1961) found that there was a midday closure of stomata at high air temperature during dry season in Nigeria. A similar result was also observed by Corley (1973) in Malaysia. Some previous studies in other crops observed that potassium deficiency led to a lower stomatal aperture. Research on alfalfa (Cooper et al., 1967) and maize (Peaslee & Moss, 1966) showed that K sufficient plants have a wider stomatal aperture than K deficient plants. In oil palm, no study has been conducted to observe whether K has influence on widening the stomatal aperture. Corley and Mok (1972) expected that severe K deficiency would influence the stomatal opening of oil palm.

Another common physiological consequence of drought stress is the production of ROS. Angelopoulos et al. (1996) reported that water stress could damage the reaction center of photosystem II (PS II) in olive trees indicated by the lower value of photosynthetic efficiency (φPSII). A similar result was reported by Suresh et al. (2010) and Cao et al. (2011) in oil palm seedling. The damage of reaction center is suspected to be caused by the activity of ROS produced during water stress as reaction center of PS I and PS II are the major generation site of ROS (Asada, 2006). As mentioned earlier, potassium has a role in eliminating ROS and thus avoid the damage of PS I and II reaction center.

In this research, we tried to discover whether potassium has any influence in widening the stomatal aperture of oil palm. We expected that there will also be a midday closure of stomata in our trial site in South Sumatra because Comte et al. (2012) reported that South Sumatra is one of the locations in Indonesia where yield-limiting water deficits of oil palm may occur during dry season. We also tried to observe whether K deficiency affect the PSII reaction center of 6-year-old oil palm by comparing the chlorophyll fluorescence of K sufficient and K deficient palms.

1.4 Research Questions and Hypotheses

The research questions were:

(1) Does potassium deficiency coupled with drought stress result in lower growth of oil palms?
(2) Does potassium deficiency coupled with drought stress result in lower stomatal conductance of palms during the day especially during midday?
(3) Does potassium deficiency coupled with drought stress result in lower photosynthetic efficiency (φPSII) and higher theoretical non-photochemical quenching (NPQ(t))?

With the following hypotheses:

(1) Palms that are potassium deficient and experience drought stress have lower leaf area and frond length than palms that are potassium sufficient
(2) Palms that are potassium sufficient and experience drought stress have a larger stomatal conductance than palms that are potassium deficient and experience drought stress
(3) Palms that are potassium deficient have lower φPSII and higher NPQ(t) than palms that are potassium sufficient in period of drought stress (as an indication of damaged PSII reaction center)
2. Material and Methods

2.1 Research Area Description

The site was located at North Musi Rawas Regency, South Sumatra about 300 km from Palembang (Figure 2). The climate was humid tropical, classified as type C1 (Oldeman classification), with an average of 6 wet months and 1 dry month per year. The yearly precipitation was around 2571 mm with a rainy season in November until April. The graphs below (Figure 3) showed the average monthly rainfall and average temperature throughout 2017 recorded at the Meteorology Station of Sultan Mahmud Badaruddin II which was the nearest climate station to the site. The site was part of the fertilizer trial of Bah Lias Research Station owned by PT. London Sumatera Indonesia. The type of oil palm crops was tenera grown in a clay loam soil.

![Figure 2. Musi Rawas Utara Regency (source: google map)](image)

2.2 Experimental Design and Description of Treatments

This study was based on a Randomized Complete Block Design (RCBD) of a current fertilizer trial of Bah Lias Research Station, PT London Sumatera, South Sumatera. We used two different plots which were Plot (+)K which had a fertilizer rate of 1.8 kg nitrogen (N) applied as urea, 0.5 kg phosphate (P) applied as rock phosphate, 2 kg potassium (K) applied as muriate of potash (MOP), and 0.3 kg magnesium (Mg) applied as dolomite per tree per year and Plot (-)K which has fertilizer rate of 1.8 kg nitrogen (N) applied as urea, 0.5 kg phosphate (P) applied as rock phosphate, 0 kg potassium (K), 0.3 kg magnesium (Mg) applied as dolomite per tree per year. Each plot had 48 palms of four varieties namely Var. A (Deli x AVROS), Var. B ((CBP6 x Deli)) x AVROS), Var. C ((Bamenda x Elite Tenera) x AVROS), Var. D ((Bamenda x Elite Tenera) x Ekona). All the palms were 6 years old.
Figure 3. Average monthly rainfall and temperature in 2017 (source: bmkg.go.id)

### 2.3 Sample Selection

Four palms were randomly sampled from each variety so that we had sixteen samples per type of plot. From each palm sample, six leaflets in the middle part of the frond number 25 were used for the measurements of stomatal conductance and chlorophyll fluorescence. Frond #25 was selected because it was the youngest frond within reach. The leaflet position was selected based on a pilot study (see result). In the pilot, we measured different fronds and different positions of the leaflets of 12 three-year-old palms (see Table 3 and Table 4). We also did a pilot to compare the φPSII and stomatal conductance measurement result between leaflets of frond #17 and frond #25 of 5 six-year-old palms.
Table 3. Frond and leaf measurement aspects compared in the pilot for φPSII measurement

<table>
<thead>
<tr>
<th>Frond Part</th>
<th>Measurement Time</th>
<th>Frond Tilt</th>
<th>Frond Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tip</td>
<td>Morning</td>
<td>Lower Tilt</td>
<td>Frond #1</td>
</tr>
<tr>
<td>Middle</td>
<td>Afternoon</td>
<td>Higher Tilt</td>
<td>Frond #9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Frond #17</td>
</tr>
</tbody>
</table>

Figure 4. Frond tilt and leaflet layers description (top layer leaflet: leaflets that grow toward top direction, down layer leaflet: leaflets that grow toward down direction)

Table 4. Frond and leaf measurement aspects compared in the pilot for $g_s$ measurement

<table>
<thead>
<tr>
<th>Leaflet Layer</th>
<th>Frond Tilt</th>
<th>Frond Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Top Layer</td>
<td>Lower Tilt</td>
<td>Frond #17</td>
</tr>
<tr>
<td>Down Layer</td>
<td>Higher Tilt</td>
<td>Frond #25</td>
</tr>
</tbody>
</table>

2.4 Parameters Measured

There were 6 parameters measured in this experiment which were stomatal conductance ($g_s$), photosystem II efficiency (φPSII), theoretical non-photochemical quenching (NPQ(t)), chlorophyll content, estimated leaf area, and frond length. Furthermore, supporting data like light intensity and quality data, temperature data, time and location data, climate and weather data, air humidity, soil water potential, and vapour pressure deficit (VPD) were also recorded from the plantation record, multispeq reading, porometer reading, and tensiometer reading (see measurements method and appendix 1 for details).
2.5 Measurements

The φPSII and NPQ(t) measurement were conducted using MultispeQ v1.0 (www.photosynq.org), while the stomatal conductance (gs) measurement was conducted using the AP4 Porometer Delta-T (www.delta-t.co.uk). Both φPSII and gs measurement were conducted by clamping the device into the sample leaflets. PAR and chlorophyll content recorded at each leaflets using multispeq at the same time of chlorophyll fluorescence measurement. In this research, the height of frond 25 was about 4-5 meters, so we climbed a portable stair until the leaflets were within reach and ensured that we can measure the leaflets without changing its position or angle. For measuring the effect of time of day (different temperature and PAR) on stomatal conductance and chlorophyll fluorescence, the measurement was conducted at three different timeslots which were morning period (8.00 am to 9.30 am), transition period (10.00 am to 11.30 am) and afternoon period (12.00 pm to 01.00 pm).

Regarding the growth parameters, frond length and frond number of each sample palms were recorded, while the leaf area was estimated by measuring the Petiole Cross Section (PCS) using calipers and measurement tape (metre). The estimated leaf area was derived from the formula described by Gerritsma and Soebagyo (1999):

\[ L = 0.35 + 0.30 \text{ PCS} \]  

*Equation 1*

where L is estimated leaf area expressed in cm\(^2\) and PCS is petiole cross section (width x depth) expressed in cm\(^2\).

The soil water potential was measured using a polymer tensiometer created by van Der Ploeg et al. (2010). Unlike the conventional tensiometer, the polymer tensiometer is able to directly measure the soil metric potentials until the theoretical wilting point (-1.6 MPa). A sixty centimeters deep hole was drilled into the soil in the active rootzone of oil palm (Omoti et al., 1983). The ceramic tip of the tensiometer was then placed in the hole. The data of soil water potential was recorded every five minutes and kept in a data logger. At the last day of observation, the tensiometer was taken out and the data was read using computer software Keller Data Logger.

2.6 Statistical Analysis

SPSS was used for the analysis of variance and fitting linear regression model. Removal of outliers was conducted based on automatic identification of outliers by SPSS. Paired t-test was used to determine statistical difference between paired objects assuming normal distribution. ANOVA analysis was conducted to determine statistical difference between treatment and plant material on the estimated leaf area, frond length, and relative chlorophyll content. ANCOVA analysis was conducted to determine a statistical difference between plots and among varieties on the stomatal conductance controlling for temperature and light intensity (PAR). The stomatal conductance was modeled as:

\[ g_s = \alpha (p) + \beta (v) + T + \text{par} + (T \times \text{par}) + \varepsilon \]  

*Equation 2*

where \( g_s \) is stomatal conductance, \( p \) is plot type, \( v \) is variety, \( T \) is temp, and \( \text{par} \) is photosynthetically active radiation (PAR), \( T \times \text{par} \) is the interaction of temperature and PAR.
and ε is the residual error that cannot be explained by the model. ANCOVA was also used to determine statistical difference between plots and among varieties on the PSII efficiency controlling for PAR. The PSII efficiency was modeled as:

$$\Phi_{II} = \alpha (p) + \beta (v) + \text{par} + \varepsilon$$  \hspace{2cm} \textit{Equation 3}

where $\Phi_{II}$ is PSII efficiency, $p$ is plot (potassium treatment), $v$ is variety, and par is PAR and ε is the residual error that cannot be explained by the model.
3. Results

3.1 Frond and Leaflet Position

3.1.1 φPSII Measurement

To select a representative sample for this research, we did a pilot measuring the φPSII of leaflets in different frond numbers, different parts of the frond, different frond tilts, and different times of the day. The leaflets among different frond numbers (including leaflets of different frond parts and tilts) showed a non-significant difference of φPSII (figure 5, upper-left panel). In contrast, leaflets located at the tip of the frond (frond 1, 9, and 17) had significantly lower φPSII than those at the middle part (tip: 0.452, middle: 0.488; t(131) = -4.12, p<0.05; see figure 5, upper-right panel)). The φPSII measured on leaflets at higher frond tilt was also significantly lower than those measured at the lower frond tilt (higher: 0.459, lower: 0.426; t(159) = 3.25; p<0.05; see figure 5, lower-left panel). Furthermore, morning measurements resulted in higher φPSII than measurements in the afternoon (morning: 0.454, afternoon: 0.350; t(47) = 4.78, p<0.05; see figure 5, lower-right panel).

![Figure 5](image)

Figure 5. Mean comparison of φPSII measured at different fronds, different leaflet location, different frond tilt and different time of the day of 3-year old oil palms (*=significant difference at p<0.05; error bars represent 95% confidence intervals)
3.1.2 Stomatal Conductance Measurement

Another pilot was conducted to assess the measurement result of stomatal conductance on leaflets at different layers and different frond tilts (only leaflets at the middle part of frond). The leaflets measured on the top layer of the frond showed significantly higher stomatal conductance (mmol m\(^{-2}\) s\(^{-1}\)) than those at the down layer (top layer: 279, down layer: 207; \(t(23) = 3.12, p < 0.05\); see figure 6, left panel). In contrast, stomatal conductance of leaflets measured at higher and lower tilt seemed to have a non-significant difference (figure 6, right panel).

![Figure 6](image)

Figure 6. Mean comparison of stomatal conductance measured at different leaflet layers, different frond tilt of 3-year old oil palms (*=significant difference at p<0.05; error bars represent 95% confidence intervals)

3.1.3 φPSII and Stomatal Conductance Measurement of 6-year old Palms

We also did a pilot measuring φPSII and stomatal conductance of 6-year-old palms at two different frond numbers which were 17 and 25. The stomatal conductance at frond 17 had a significantly higher stomatal conductance than at frond 25 (leaf 17: 471, leaf 25: 358; \(F(44) = 6.309, p < 0.05\); figure 7, left panel). Meanwhile, the φPSII observed at both frond number did not show a significant difference.

![Figure 7](image)

Figure 7. Mean comparison of stomatal conductance and φPSII measured at leaflets of frond number 17 and 25 of 6-year old palms (*=significant difference at p<0.05; error bars represent 95% confidence intervals)
Based on the pilot, we found out that leaflets at the top layer of the middle part of frond 17 as the most representative sample. However, as frond 25 was the only frond within reach for measurement in 6-year old palms, we decided to measure leaflets of the top layer of the middle part of frond 25 both at higher and lower tilt in the actual experiment.

### 3.2 Growth Parameters and Light Intensity (PAR)

To assess leaf area and vegetative growth, we measured frond length and PCS (Petiole Cross Section) of all sample palms. The palms in the (-)K plot had a significantly larger leaf area (m²) compared to palms in the plot (+)K (plot (-)K: 7.6; plot (+)K: 6.1; F(1, 24) = 16.6, p < 0.05; figure 8, upper-left panel). A significant difference also existed among varieties (F(3, 24) = 6.6, p < 0.05). Variety C had significantly lower leaf area compared to other varieties (see figure 8, upper-right panel). Similarly, in terms of frond length, palms in the (-)K plot had significantly longer fronds than palms at the (+)K plot (F(1, 24) = 16.5, p < 0.05; figure 8, lower-left panel). A significant difference was also observed among oil palm varieties with variety C having significantly shorter fronds than the other varieties (figure 8, lower-right panel). This result implied that variety C ((Bamenda x Elite Tenera) x AVROS) showed less vigorous vegetative growth than other varieties. Furthermore, because of the smaller LAI (Leaf Area Index), the PAR recorded at frond 25 in plot (+)K was higher than in plot (-)K (figure 9).

![Graph showing comparison of leaf area and frond length](image)

*Figure 8. Mean comparison of estimated leaf area and frond length of four varieties of oil palm grown under different fertilizer treatment (*=significant difference at p<0.05; means followed by the same letter are not significant at p<0.05; error bars represent 95% confidence interval)*
Figure 9. Mean comparison of PAR between plots and at different timeslot (*=significant difference at p<0.05; error bars represent 95% confidence intervals)

3.3 φPSII, NPQ(t), and Relative Chlorophyll Content

Using the multispek, we measured several parameters of photosynthesis: φPSII, NPQ(t), and relative chlorophyll content. We did not observe a significant difference between plots and among varieties in terms of chlorophyll content (Table 5).

Table 5. Relative chlorophyll content of oil palm varieties grown under different fertilizer treatment

<table>
<thead>
<tr>
<th>Plot</th>
<th>Relative Chlorophyll Content (SPAD Units)</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>Plot (+)K</td>
<td>72.9 ns</td>
<td>67.9 ns</td>
</tr>
<tr>
<td>Plot (-)K</td>
<td>70.8 ns</td>
<td>74.7 ns</td>
</tr>
<tr>
<td>Mean</td>
<td>71.8 ns</td>
<td>71.3 ns</td>
</tr>
</tbody>
</table>

ns = non-significant

However, we observed the effect of fertilizer treatment and variety on PSII efficiency after controlling for PAR. Palms in the (-)K plot had a significantly higher φPSII than those in the (+)K plot ((+)K = 0.432, (-)K = 0.403; plot: F(1, 27) = 4.7, p < 0.05; see figure 10; upper-left panel). While regarding the NPQ(t), palms at plot (+)K had a significantly higher φPSII than palms at plot (-)K (figure 10; lower panel). In terms of varieties, variety D showed the highest φPSII but it was non-significantly different from variety B and A. Meanwhile, variety C showed significantly lower φPSII and the lowest among four varieties (Figure 10; upper-right panel).
Figure 10. φPSII and NPQ(t) of four varieties of oil palm grown under different fertilizer treatment (*=significant difference at p<0.05; means followed by the same letter are not significant at p<0.05; error bars represent 95% confidence intervals)

3.4 Air Humidity, Soil Water Potential, Vapour Pressure Deficit, and Stomatal Conductance

The soil water potential data showed that the soil was quite saturated with pF ranging from 2.02 to 2.72, but with a decreasing pattern from the first day of measurement until the last day of measurement as rainfall ceased over time (figure 11, right panel). No acute water shortage occurred during the study, but the air humidity recorded during the day showed a significant decreasing trend from morning to afternoon (F(2,93) = 44.4, p<0.05; figure 11, left panel). The measurement result of stomatal conductance at different timeslots showed a significant response to air drying at palms of plot (+)K but not at the palms of plot (-)K (figure 12). Palms at plot (+)K showed a significantly lower stomatal conductance during the afternoon period while palms at plot (-)K did not show a significant decrease in stomatal conductance from morning to afternoon. We also noticed that palms at plot (-)K had a high variability of stomatal conductance in the morning period and not as high in the transition and afternoon period. In figure 13, we showed that in the morning period palms at plot (-)K had a higher variability and also that majority of the stomatal conductance value was in the lower half of the range than at the higher half. On average, stomata in the (+)K plot seemed to be more open during the transition period compared to those in the (-)K plot, but this result was not significant. In terms
of variety, we also observed a non-significant effect of variety on the stomatal conductance after controlling for temperature and PAR.

Figure 11. Air relative humidity at different timeslots and soil water potential (pF) at different measurement dates (means followed by the same letter are not significant at p<0.05; error bars represent 95% confidence intervals)

Figure 12. Vapour Pressure Deficit (VPD) and stomatal conductance of oil palm at different timeslot in each plot (means followed by the same letter are not significant at p<0.05; error bars represent 95% confidence intervals)
Figure 13. Stomatal conductance of palms of plot (-)K and (+)K at different timeslots
4. Discussion

As a preliminary study, this thesis provided insights about the methodology of measuring stomatal conductance and chlorophyll fluorescence of 6-year old oil palms in field conditions. In the pilot, we were able to observe the difference of measurement results conducted at leaflets of different fronds and at different parts of the frond. We found that the photosynthetic efficiency (φPSII) among different frond numbers were not significantly different. In the actual experiment, even though we found a bias in our growth and chlorophyll fluorescence result due to improper management of the trial site in the past, we did observe midday closure of oil palm stomata during the rainy season. We also observed a clear difference of midday closure between the palms at plot (+)K and palms at plot (-)K.

4.1 Frond and Leaflet Position (Pilot Study)

4.1.1 φPSII Measurements

In oil palm, the older fronds (indicated by higher frond number) are always located below the younger fronds which means that older fronds are always shaded by younger fronds. In the pilot, we measured φPSII of 3-year-old palms to determine the variability of measurement conducted on leaflets of different frond numbers and at different parts of the frond. Corley (1983) reported that there was no significant difference in photochemical efficiency among frond ages (frond 4 to 36) of 3-year-old oil palm. In contrast, another study by Henson (1991a) reported a significant difference in photochemical efficiency between younger fronds (frond 2-8) and older fronds (frond 31-39) of mature palms. In our research, we observed a non-significant difference of φPSII among different frond numbers (frond 1, 9, and 17) of 3-year-old palms. Beside similar to what Corley (1983) found, our finding was also in agreement with Haniff et al. (2005) who observed a non-significant difference of φPSII among frond 1, 9, and 17 of 17-year-old palms. We did not measure the frond number 25 of 3-year-old palms, however, we measured the φPSII of frond 17 and 25 of 6-year-old palms and we found that the difference was not significant even though the frond 25 started showing senescence symptom. Studies in strawberry (Keutgen et al., 1997) and wheat (Nesterenko et al., 2015) also reported that photochemical efficiency remains the same with increasing leaf age.

One possible hypothesis that explains why senescence leaves have the same photosynthetic efficiency as younger leaves was proposed by Adams et al. (1990). They argued that leaf senescence is a controlled process after finding a non-significant difference of φPSII between young leaves and senescence leaves of Platanus occidentalis (American planetree). Their hypothesis was supported by the finding of Jenkins et al. (1981) in bean. Based on our result, we proposed that oil palms may have the same physiological characteristic of American Planetree and bean. Further physiological study of leaf senescence process in oil palm would be needed to prove this hypothesis. The non-significant difference of φPSII between frond ages implied that the level of contribution of assimilation between frond numbers in oil palm is determined by the level of PAR received by each frond. The younger frond must have a higher assimilation than older frond as it receives higher PAR. This was confirmed by the study of Corley (1983), Haniff et al. (2005), and Henson (1991b).

Regarding the difference between palm ages, we observed that φPSII measured at 6-year-old palms were slightly higher than 3-year old palms at 0.55 and 0.50 respectively. Suresh et al. (2010) and Suress et al. (2012) measured φPSII of 10-month palms seedling and the value of φPSII was at about 0.60 while Cha-Um et al. (2010a) and Cha-Um et al. (2010b) measured φPSII of palm seedlings developed from a tissue culture and observed a φPSII value of 0.52.
Moreover, Haniff et al. (2005) recorded that the φPSII of 17-year-old oil palm was at about 0.73. A study of Apichatmeta et al. (2017) found that the φPSII of adult (5 to 10-year-old palms) and juvenile (6-month-old palms) were not significantly different at about 0.62. These findings implied that palms age had no correlation with the value of φPSII. The cause of variation was probably the genotype of the palms (as what we also observed in this study; see figure 10, upper-right panel), the environment especially related to the level of PAR, and the condition of the palm whether it is stressed or not. Stressed palms usually have lower φPSII (Murchie & Lawson, 2013). The φPSII value that we observed in this research was quite low in comparison to previous studies which indicated that we measured stressed palms.

Using oil palm seedlings for the φPSII measurement has some advantages. First, by measuring seedlings, it is easy to reach frond 1 and we can also measure the linear electron flow (LEF). Unlike φPSII, LEF is only comparable when fronds being measured receive the same intensity of PAR. Hence, to compare plots, this can only be achieved if we measure frond 1 as it is always unshaded. Second, by measuring seedlings we can avoid measuring senescence leaflets. However, seedlings also have several disadvantages. First, palm seedling may have a significantly different physiology than mature trees like what has been found by Apichatmeta et al. (2017) who found that there were different photosynthetic characteristics such as ETR (Electron Transport Rate) between juvenile and adult oil palms. Second, seedling measurements were usually conducted in a controlled environment like a greenhouse so that it may not fully represent the actual condition faced by palms in the field conditions. For instance, palms in a greenhouse do not receive full natural light since greenhouse only transmits a portion of natural radiation. Henson (1991a) reported that palms grown in the field and in the greenhouse had different physiological characteristics in terms of low light compensation points and dark respiration rates.

Regarding leaflets at different parts of the frond, a significantly higher φPSII and lower variability of leaflets at the middle part of the frond than the tip part might be related to the quite uniform amount of PAR received by the middle part compared to the tip part. We observed that the tip frond part received a varying amount of PAR as some tips located in the outer part of the canopy while others were located inside of the canopy. Higher variability of on the frond tip leaflets might also be due to the small measurement area which made repeated measurements (measurement without changing leaf natural position) hard to be done. Hence, also for practical reason, the middle part was better for doing the measurements. Some previous research by Gerritsma (1988) and Haniff (2006) also used the middle part of the frond to measure net photosynthetic rates. Different level of absorbed PAR might also be the reason for the significantly higher φPSII of higher frond tilt when compared to lower frond tilt (see the description of frond tilt in figure 4) and the significantly higher φPSII in the morning than in the afternoon.

Based on the pilot, to measure φPSII on mature oil palm trees in the field, the most representative leaflets are those at frond 17 as it usually does not show stress and senescence symptom when the palm is stressed. Frond 1 would also be ideal, but we highly doubt that it is possible considering the acute angle of frond 1 which makes a proper measurement with multispeq impossible. Next, leaflets in the middle part of frond are more preferred because it is uniformly shaded and always have larger leaf areas than leaflets at the tip. Measuring both frond tilts and measurement at several times during the day would also be ideal to get a representative data of leaflets that received different PAR. Kuhlgert et al. (2016) stated that the result of the φPSII measurement is very dependent on the level of PAR.
4.1.2 Stomatal Conductance Measurement

The structure of oil palm frond is such that leaflets can be divided into two layers which we defined as top layer and down layer (figure 4). Higher stomatal conductance showed by leaflets at the top layer than the down layer might be related to the different level of shade received between both. In terms of different stomatal conductance between frond ages, a higher stomatal conductance for leaflets of frond 17 (upper frond) than frond 25 (lower frond) might be related to the differences in absorbed PAR. The frond 17 of 6-year-old palms were usually under half shade while the frond 25 was usually under full shade. The conversion factor was:

\[ g_s \text{ of frond #17} = 0.405 \times (g_s \text{ of frond #25}) + 325.95 \]  

where \( g_s \) is stomatal conductance (mmol m\(^{-2}\) s\(^{-1}\)) and the R\(^2\) (correlation) was 0.201. Another possible cause was the different frond age. Henson (1991b) reported that in mature palms the stomatal conductance of frond 3 was consistently higher than frond 6. However, Dufrene and Saugier (1993) and Haniff (2006) showed that frond ages in mature palms did not affect stomatal conductance. The difference that we observed in our result might be caused by the senescence of frond 25.

![Figure 14. Description of senescence at frond number 25](image)

Regarding the palm age, we observed that the stomatal conductance of 6-year-old palms and 3-year-old palms were not different, in line with the conclusion of Dufrene and Saugier (1993). We proposed that palms age did not have a significant effect on the stomatal conductance \( \varphi_{PSII} \). However, under drought stress condition, palm age might have an effect. For example, unlike young palms, mature palms have a larger water and nutrient availability as a result of greater root exploitation. Thus, it is expected that older palms might have a larger stomatal conductance than younger trees during drought stress.
Based on the pilot of stomatal conductance measurement, the ideal frond number for a stomatal conductance measurement is frond number 17 for the same reason mentioned in the chlorophyll fluorescence pilot discussion. However, as what we have experienced, sometimes frond 17 was just too high or at a position where a proper measurement with porometer was difficult. In that case frond 25 and we followed the approach of Corley (1973) who measured leaflets at frond 24-36 (lower half fronds) of mature oil palm in the field conditions. He argued that the variation in stomatal resistance with leaf age was relatively slight. Regarding the frond part, we selected the middle part of the frond as it is usually uniformly shaded and has a larger leaf area which will make the measurement easier. Leaflets at the middle part of the frond have also been used in some previous research by Gerritsma (1988) and Haniff et al. (2005). In terms of frond tilt, measuring either one of them or both would be equally good as there was no significant difference between both tilts.

4.2 Growth Parameters and Light Intensity (PAR)

Corley and Mok (1972) reported that potassium caused an increase in leaf area of oil palm. Thus, we expected palms at (+)K plot to have larger leaf area and longer fronds than palms at (-)K plot. However, our result showed that palms at (-)K plot had a significantly larger leaf area and longer frond length than palms at (+)K plot which indicated that palms at plot (-)K had a more vigorous vegetative growth. There were some possible causes of this result but we suspected that the most possible cause was lack of maintenance especially weeding in the plot (+)K. Based on our interview with the staff, there was a possibility that the plot (+)K received less proper maintenance than the plot (-)K but the fertilizers were properly applied at both plots. The area of the trial site was under dispute between company and locals for the past few years, thus the maintenance of the trial could not be optimal as it was abandoned for several months when the conflict got worse. Furthermore, the plot (+)K was located in an area where a proper maintenance would require more effort from the labor and where it was hard for supervisors to do regular checking. Meanwhile, plot (-)K had a close proximity to the main road which made regular checking easy and made it requires less effort for maintenance. When we presented the data that palms at plot (+)K had lower growth, the supervisory staff was also surprised which means that they barely check this plot. If they visited it often then they might easily notice that palms at plot (-)K had better growth than palms at plot (+)K. Improper weeding of the plot (+)K site led to rapid growth of weeds surrounding the palms and may have resulted in resources competition. The competition was probably even more fierce in the immature phase as there was also light competition between palms and weeds. This made the palms at plot (+)K experienced stress and had less vigorous vegetative growth. The higher PAR level recorded on frond 25 at plot (+)K implied that the level of PAR interception of palms canopy at plot (+)K was lower than palms at (-)K which may result in a lower total photosynthesis rate and lower yield. However, higher PAR was not followed by higher temperature which resulted in a non-significant difference of vapour pressure deficit (VPD) between both plots.

Regarding the growth comparison among varieties, we observed that palms of variety C which were the progenies of crossing between (Bamenda x Elite Tenera) and AVROS showed significantly lowest leaf area and frond length. The best growth was shown by the variety D which were the progenies of crossing between (Bamenda x Elite Tenera) and Ekona even though the difference with the variety A (Deli x AVROS) and B (CBP6 x Deli) was not significant. Compared to the finding of Gerritsma and Soebagyo (1999), conducted at the same company, the average leaf area of the cultivar in this research was slightly smaller than the cultivar that they used 18 years ago. Hardon et al. (1969) showed that in oil palm there was a
positive correlation between leaf area and yield. Thus, we could expect that palms at plot (+)K have a lower yield than palms at plot (-)K and that palms of variety C ((Bamenda x Elite Tenera) and AVROS) have a lower yield than palms of other varieties when light is the limiting factor.

4.3 φPSII, NPQ(t), and Relative Chlorophyll Content

Operational quantum yield efficiency (φPSII) and non-photochemical quenching theoretical (NPQ(t)) data have been proven to be useful in determining whether plants experience substantial photoinhibition or down-regulation of PSII due to long-term or severe stress like drought stress. In our research, there was a possibility of drought stress experienced by the palms during dry season if we look into the climate data of 2017 (Figure 3). Moreover, Comte et al. (2012) had reported that South Sumatra is one of the areas in Indonesia where dry season could limit the yield of oil palm crops. We observed a significant difference of φPSII and NPQ(t) between plots while there was a non-significant difference of relative chlorophyll content between plots and varieties. However, in opposite to our hypothesis, φPSII of palms at plot (+)K were significantly lower, while its NPQ(t) were significantly higher than palms at plot (-)K.

There is no arbitrary line of φPSII value to classify whether palms are stressed or not. Previous studies usually compared the stressed palms with control and always observed that stressed palms had a significantly lower φPSII and higher NPQ than the non-stressed palms (Suresh et al. (2012); Suresh et al. (2010); Haniff et al. (2005); Cha-um et al. (2012); Cha-Um et al. (2010a); Cha-um et al. (2010b)). In our case, the result showed that the control (plot (+)K) turned out to have a lower φPSII. Assuming that palms at plot (-)K were stressed due to potassium deficiency, our result indicated that palms at plot (+)K were more stressed than palms at plot (-)K. However, as there was limited information regarding the leaf potassium content and we were unable to assess our assumption that plot (-)K experienced potassium deficiency. We proposed that the strange result that we had was due to the same reason of improper maintenance of the trial site as what has been previously explained. However, assuming that the fertilizer was correctly applied (based on the interview result) and that the basal potassium level of plot (-)K was not adequate, we proposed that palms at both plots were stressed for different reasons. Palms at plot (-)K were stressed due to potassium deficiency as indicated by the symptom of potassium deficiency on leaflets while the palms at plot (+)K were stressed from resource competition with weeds.

In terms of varieties, we observed that palms of variety C (Bamenda x Elite Tenera) and AVROS showed a significantly lower φPSII and were the lowest among four varieties. The highest was shown by variety D (Bamenda x Elite Tenera) and Ekona even though the difference with the variety A (Deli x AVROS) and B (CBP6 x Deli) was not significant. This result was similar with the result of growth measurement which implied that the φPSII value of palms may have a close relationship with palms growth as what has been observed by Cha-Um et al. (2010a) and Cha-um et al. (2012). The more efficient photosystem II, the higher vegetative growth of palms could be achieved. Furthermore, yield of oil palms is considered to be source-limited (Henson, 1991b), therefore, selection based on higher photosynthetic rates may be useful to get high-yielding variety. In our case, variety D (Bamenda x Elite Tenera) x Ekona) was the most promising high-yielding variety under stress.
4.4 Air Humidity, Soil Water Potential, Vapour Pressure Deficit (VPD), and Stomatal Conductance

The midday closure was always indicated by significantly lower stomatal conductance during the day. Gerritsma (1988) recorded a midday closure during dry season in Papua New Guinea and expressed the stomatal conductance in mm/s. If we converted the data into mmol m$^{-2}$ s$^{-1}$ by multiplying it with 41 (Korner, 1979), during midday closure the stomatal conductance was lower than 410 mmol m$^{-2}$ s$^{-1}$ from about 800 in the morning. In India, Kallarackal (1996) recorded that the maximum stomatal opening during the dry season was about 500 mmol m$^{-2}$ s$^{-1}$ maximum and during midday, it was about 100 mmol m$^{-2}$ s$^{-1}$ or lower. In this study, we observed that palms at plot (+)K showed a decreasing trend of stomatal conductance and a significantly lower stomatal conductance in the afternoon which was the indication of midday closure. Meanwhile, palms at plot (-)K showed a declining trend of stomatal conductance as well but the stomatal conductance in the afternoon was not significantly lower. Rees (1961) and Corley (1973) reported that midday closure happened during dry season. As the measurement was not conducted under drought stress as what we have expected before the experiment, our results indicated that midday closure also happened during rainy season when the VPD during the day was increasing but the soil was well-watered which confirmed the finding of Smith (1989). This finding was also similar to what has been reported by Dufrene and Saugier (1993) who observed that increase in VPD was followed by a rapid stomatal closure even though the soil was well-watered.

We hypothesized that potassium deficiency would result in a lower stomatal conductance of palms, especially during midday. While we observed a symptom of potassium deficiency at palms of plot (-)K (figure 15), we did not prove that palms with potassium deficiency had a lower stomatal conductance, however, we observed that some potassium deficient palms showed a lower stomatal conductance in the morning period. Our result showed that the midday closure only happened at palms with sufficient potassium and not at palms with potassium deficiency. However, if we compared the value of stomatal conductance between plots in the afternoon, we proposed that palms at both plots were actually having midday closure but because of the high variability of stomatal conductance value of palms at plot (-)K in the morning, the difference between timeslots was not significant. If we compare the level of variability between morning period and another period, we noticed that the high variability only happened in the morning and that the value of stomatal conductance was concentrated on the lower half of the range (see figure 13). Based on that observation, we proposed that some palms at plot (-)K had a reduced ability to open its stomata fully in the morning which might be caused by potassium deficiency. This might be possible as we also observed a varying degree of potassium deficiency between palms at plot (-)K indicated by the level of potassium deficiency symptom they showed. Further study observing the stomatal opening of potassium deficient palms compared to potassium sufficient palms using microscope would be useful to prove this hypothesis.
Figure 15. Leaflets of palms at plot (-)K showing potassium deficiency symptom

Humble and Hsiao (1970) reported that increment of K⁺ in the guard cell causes the opening of stomata. This implied that under potassium deficiency the opening of stomata will be inhibited. Our results were similar with studies in spinach (Tomemori et al., 2002) and wheat (Dhakal & Erdei, 1986), showed that K deficiency favored stomatal closure. Beside related to the low K⁺ ion in the guard cell, one other possible reason for this was that potassium deficiency led to poor root growth of palms which influenced the water absorption of some palms and resulted in low stomatal conductance during morning period. Martineau et al. (2017) stated that sufficient K supply promotes root growth which contributes to higher water uptake by improving deep root exploration. Having said that potassium deficiency might favor stomatal closure, some other studies in olive trees (Arquero et al., 2006), sunflower (Lindhauer, 1985), and cotton (Bednarz et al., 1998) reported that K deficiency might favor stomatal opening. Moreover, some other research in pearl millet (Ashraf et al., 2001), almond trees (Basile et al., 2003), and alfalfa (Peoples & Koch, 1979) even showed that potassium has no effect at all on the stomatal mechanism. Beside possible differences among species, another possible hypothesis that explained this variability was proposed by Hsiao and Lauchli (1986). They argued that many studies about the relation between K deficiency and stomatal conductance were conducted at a very advanced stage of K deficit. At this stage, other metabolic processes might have been affected which could modify the initial stomatal response to K deficiency. In our research, there was a possibility that other metabolic processes were affected.

Regarding the varieties, a non-significant difference that we observed indicating that all varieties that we had in our research did not differ in terms of stomatal conductance. This implied that the genetic potential of each variety that we had was not different in terms of stomatal opening trait. In contrast, previous study showed that type of varieties or planting material could have a different trait of stomatal opening (Méndez et al., 2012). However, on that study, the stomatal conductance difference was ranging from 118 to 148 mmol m⁻² s⁻¹, while in our study it was ranging from 328 to 411 among varieties. Considering the higher range and the magnitude of variability that we had, we proposed that the non-significant difference that we observed among varieties might also due to the relative small sample size.
5. Conclusion and Recommendation

Our research has provided insights in the difference between φPSII and stomatal conductance measurements of different fronds, different parts of the frond, and different locations of leaflets. We observed that different frond numbers had a quite similar value of φPSII as leaf senescence in oil palm might be a controlled process that does not reduce the photosynthetic efficiency. In contrast, leaf senescence did affect the stomatal conductance. Based on the pilot, we concluded that leaflets at the middle part of the frond 17 as the best leaflets to be measured for young and mature palms. In the actual experiment, even though we had a biased result of growth and φPSII due to improper management of site trial, we observed an important information that variety C ((Bamenda x Elite Tenera) and AVROS) has a lower performance in terms of growth and φPSII compared to the other varieties under stress condition. This research also found the different stomatal behavior of palms between different potassium treatment. The difference might due to a small sample size that we had, however, as it only happened in the morning, there was a possibility that some palms at plot (-)K had a reduced ability to fully open its stomata in the morning.

As a preliminary study, our findings give an important information for anyone who wants to do field observations on palms in the future. Replication and a larger sample size is recommended to reduce the variability and to be able to conclude on the cause of the difference in stomatal behavior. Having a well-maintained trial, predawn Fv/Fm data and complete information about soil and leaves data would also be important to support any findings that may be found in the future research. Research about the level of potassium ion in the guard cell of oil palm during drought stress would be interesting to prove that potassium deficiency influences the stomatal behavior of oil palm. Furthermore, regarding the similar photosynthetic efficiency in a senescence leaf, future physiological study of senescence leaf would be useful to get a better understanding about senescence process in oil palm. Finally, this study added new knowledge and a better understanding about sample selection for stomatal conductance and chlorophyll fluorescence measurement of oil palm and stomatal responses of oil palm in the field conditions.
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Appendix 1. Detailed Measurement Protocol

1.1 Chlorophyll Fluorescence Measurement

Introduction

Chlorophyll fluorescence measurement was conducted using MultispeQ v1.0 (www.photosynq.org). Multispeq is a quite new device to measure chlorophyll fluorescence and therefore it is still under development and some features are not yet reliable. For instance, the sensor for CO₂ and leaf transpiration does not deliver a precise data so that the photosynthetic rate and the leaf transpiration data is not accurate. However, the advantage of the multispeq is that you can get many kinds of data just by doing one measurement. The reliable data are φPSII, NPQ(t), and chlorophyll content, PAR, and relative humidity. This device can also be used for predawn Fv/Fm measurement. However, for predawn measurement, as not many have done it using this device and there was no specific tutorial provided by the manufacturer on how to use the device for the predawn Fv/Fm measurement, we made a mistake. Thus, we shared this detailed explanation so that this mistake would never be repeated by other users. The only multispeq protocol provided by the manufacturer is the leaf multispeq 1.0. While, for predawn Fv/Fm you need to make the protocol yourself using Java programming language as it is open source. Other alternative is to search for a protocol in the website and test whether it shows the normal value of predawn Fv/Fm.

Measurement Time and Best Measurement Practice

The φPSII is very much dependent on the PAR level. Thus, the best practice is to measure the same leaflet at two different time a day (at least) for instance morning and afternoon so that we recorded the value of φPSII at two different level of PAR. More measurement during the day is better. For predawn, as it is conducted at a dark-adapted state then a one time measurement is enough. There are two options for Fv/Fm measurement, some scientist dark adapt the leaflets by covering the leaf for 30 minutes and then measure it and some other scientist prefer to get the predawn value of Fv/Fm. The predawn is especially useful to detect the stress of the plant as it represents the maximum efficiency of photosystem II.

There are a couple of important practices that you have to be aware of to get a high quality data and also avoiding error.

1. Position yourself so that you are not shadowing the leaflets or light sensor.
2. Ensure that the light sensor experience the same ambient light level as the leaflet at its natural position therefore do not move the leaflets from its natural position. When you are climbing s scaffold then it might be a bit harder but be sure to hold the device firmly otherwise the sensor reading might be interrupted.
3. Clamp the leaf toward the leaf on the left side when facing toward the stem whenever possible. This especially important if you use the compass data.
4. Make sure that the leaves completely cover the measurement zone. If it only partially covers the measurement zone then the signal quality will suffer and will lead to an incorrect result especially about the chlorophyll content and absorbance data. If you have a small leaf consider using the mask. You can find the tutorial of making the mask at photosynq website and make the mask by yourself. Fifth, open and close the clamp as quick as possible because plants adapt to light condition in seconds or even miliseconds. A good measurement might look like the figure 15 below.
Figure 16. φPSII measurement of oil palm leaflets

φPSII, NPQ(t), and Chlorophyll Content measurement

1. At a time of day of saturated light intensity, find the sample palms
2. Select frond 25 and identify which leaflets to be measured
3. Prepare the tablet and the multispeq by connecting its bluetooth connection, log in into the project, choose the ordinary protocol (leaf multispeq V1.0) and filling up question to give an identity of the measurement
4. Carry out the measurements with the MultiSpeq by clapping it into each leaflets and wait until 15 – 20 seconds
5. Click accept in the tablet if the data looks good. If there is an error notification, discard the data and do the measurement again at the same leaflet until you get a good data.
6. Do the next measurements according to the timeslots and the measurement sequence that have been planned.

Predawn Fv/Fm measurement

1. Identify the frond and leaflets that are going to be measured and mark it with paint the day before the measurement.
2. Just before dawn, visit the plantation and find the sample palms that have been marked
3. More or less at the point where the rachis goes triangular, cut of 6 leaflets with a harvesting sickle.
4. Collect the leaflets and label them
5. Place the leaflets in a cardboard box, in the dark
6. Prepare the tablet and the multispeq by connecting its bluetooth connection, log in into the project, select the pre dawn protocol filling up question to give an identity of the measurement
7. Carry out the measurements with the MultiSpeq by clapping it into each leaflets and wait until 15 – 20 seconds
8. Measure the Fv/Fm using the MultiSpeq, in the dark environment (a windowless room with a small light)
1.2 Stomatal Conductance Measurement

Introduction

Stomatal conductance measurement was conducted using porometer AP4 Porometer Delta-T (www.delta-t.co.uk). Along with porometer you may need to bring its calibration equipment and paper and pen to record the data. Things that you need to pay attention with is the calibration of porometer. In the morning when the humidity is quite high and that we did calibration under shady condition, we needed to repeat the calibration for more than five times. There is no best way to avoid this, what we can do is to always make sure we prepare the calibration tool appropriately so that we can reduce the possibility of calibration error. Furthermore, changing the desiccant regularly might also help to reduce the chance of calibration error.

Stomatal conductance measurement

1. Prepare the calibration plate an our before conducting the calibration
2. Calibrate the porometer
3. At a time of day of saturated light intensity, find the sample palms
4. Select frond 25 and identify which leaflets to be measured
5. Carry out the measurements with the porometer by clapping it into each leaflets and wait until it shows a measurement result
6. Record the result in a paper. The data to be recorded includes the PAR, stomatal conductance, time, and temperature
7. Do the next measurements according to the timeslots and the measurement sequence that have been planned
8. Calibrate the porometer every an hour and half or two hours to ensure a precise measurement result
9. Leaf transpiration data is derived from the stomatal conductance data using the formula described by Ansley et al. (1994)
Appendix 2. Estimation Based on Data

From the data that we have we can estimate some other data such as:

1. Transpiration rate: using model of Ball et al. (1987) citing from Gerosa et al. (2012) we can estimate transpiration rate using this ball-berry-leuning (BBL) model

\[ E = 1.6 \times g_s \times VPD \]  

*Equation 4*

Where E is transpiration rate (kPa mmol/m²s⁻¹), g_s is stomatal conductance, and VPD is vapour pressure deficit.

We used the frond 25 data and multiply it with estimated leaf area for this estimation of leaf transpiration rate and as can be seen in the figure below. We observed that the transpiration rate showed an increasing trend during the day even though the difference was not significant. For palms at plot (+)K, in the morning period, the transpiration rate of palms was the lowest as stomatal conductance was the highest but the VPD was the lowest. During transition period, when the VPD increased significantly the stomatal conductance did not drop significantly so that the transpiration rate increased. In the afternoon, when again the VPD increased significantly the stomatal conductance was significantly lower which made the transpiration rate remain the same as transition period. This data implied that the stomata did not close to the level that reduces the transpiration rate so that the water use efficiency (WUE) decreased even though the assimilation was slightly higher as more CO₂ enter the stomata. This might be related with the well-watered soil that we had in this research. In contrast to what have been observed by Silva et al. (2017) who reported that during soil water limited condition the transpiration rate of oil palm decreased during the day as the VPD increasing, adequate water supply seemed to influence the stomata to not fully close during high VPD.

Meanwhile for palms at plot (-)K, in the morning period, the stomatal conductance was the highest, but the VPD was the lowest so that the transpiration rate was low. In the transition period, when the VPD increased significantly, the stomatal conductance was not significantly lower so that the transpiration rate was slightly increased. In the afternoon, the VPD increased significantly however the stomatal conductance did not decrease significantly which made the transpiration rate increased. These results gave us an information that potassium deficiency may have impaired both the stomatal opening and stomatal closing.

There was a possibility that the interpretation above might be misleading. According to Gerosa et al. (2012), this BBL model has limitation in describing the stomatal closure in a drought condition. Dewar (2002) made a better model by taking into account soil water content (SWC). He coupled the BBL model with tardieu model for stomatal response to drought by incorporating the data of dark respiration, leaf water potential, and concentration of ABA (Absicic acid). As we did not have those data, we were unable to compare the BBL model with the model developed by Dewar (2002). Thus, further research comparing Dewar model and BBL model will be very interesting.
2. If we upscale it to the tree level by multiplying the transpiration rate with average frond number of 6-year-old palms and with estimated leaf area, we can see that variety D had a slightly higher transpiration rate although it was not statistically significant. This result almost looked like the comparison of growth and φPSII of each varieties in the result data where variety C had the lowest transpiration rate. However, the difference this time was not statistically significant which might relate with the non-significant difference of stomatal conductance. Comparison between plots also showed non-significant difference.
### Appendix 3. Result Data

Table 6. Mean comparison of stomatal conductance response of oil palm varieties at different timeslots and different fertilizer treatment

<table>
<thead>
<tr>
<th>Plot</th>
<th>Timeslot</th>
<th>Stomatal Conductance (mmol m⁻² s⁻¹)</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>Plot +K</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.30 – 10.00</td>
<td>406 ns</td>
<td>451 ns</td>
<td>420 ns</td>
</tr>
<tr>
<td>10.30 – 11.30</td>
<td>311 ns</td>
<td>381 ns</td>
<td>362 ns</td>
</tr>
<tr>
<td>12.00 – 13.30</td>
<td>306 ns</td>
<td>416 ns</td>
<td>294 ns</td>
</tr>
<tr>
<td>Mean</td>
<td>341 ns</td>
<td>416 ns</td>
<td>359 ns</td>
</tr>
<tr>
<td>Plot -K</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.30 – 10.00</td>
<td>322 ns</td>
<td>467 ns</td>
<td>567 ns</td>
</tr>
<tr>
<td>10.30 – 11.30</td>
<td>418 ns</td>
<td>313 ns</td>
<td>364 ns</td>
</tr>
<tr>
<td>12.00 – 13.30</td>
<td>362 ns</td>
<td>346 ns</td>
<td>299 ns</td>
</tr>
<tr>
<td>Mean</td>
<td>367 ns</td>
<td>375 ns</td>
<td>410 ns</td>
</tr>
</tbody>
</table>

*ns = non-significant difference*

Table 7. φPSII response of oil palm varieties at different fertilizer treatment (means followed by the same letter are not significant at p<0.05; capital letter for different between plots and small letter for different between varieties)

<table>
<thead>
<tr>
<th>Plot</th>
<th>φPSII (ratio unit)</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>Plot (+)K</td>
<td>0.550 Ba</td>
<td>0.526 Aa</td>
</tr>
<tr>
<td>Plot (-)K</td>
<td>0.605 Aa</td>
<td>0.564 Ab</td>
</tr>
<tr>
<td>Mean</td>
<td>0.551 a</td>
<td>0.561 a</td>
</tr>
</tbody>
</table>
Table 8. Estimated leaf area of oil palm varieties under different fertilizer treatment (means followed by the same letter are not significant at p<0.05; capital letter for different between plots and small letter for different between varieties)

<table>
<thead>
<tr>
<th>Plot</th>
<th>Estimated Leaf Area (m$^2$)</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>Plot (+)K</td>
<td>6.0 Aa</td>
<td>6.0 Ba</td>
</tr>
<tr>
<td>Plot (-)K</td>
<td>7.8 Aa</td>
<td>8.5 Aa</td>
</tr>
<tr>
<td>Mean</td>
<td>6.9 a</td>
<td>7.3 a</td>
</tr>
</tbody>
</table>

Table 9. Mean comparison of frond length of four varieties of oil palm grown under different fertilizer treatment (means followed by the same letter are not significant at p<0.05; capital letter for different between plots and small letter for different between varieties)

<table>
<thead>
<tr>
<th>Plot</th>
<th>Frond Length (cm)</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>Plot (+)K</td>
<td>469 Ba</td>
<td>443 Ab</td>
</tr>
<tr>
<td>Plot (-)K</td>
<td>539 Aa</td>
<td>523 Aa</td>
</tr>
<tr>
<td>Mean</td>
<td>504 a</td>
<td>483 ab</td>
</tr>
</tbody>
</table>
Table 10. Petiole Cross Section (PCS) of oil palm varieties at different fertilizer treatment (means followed by the same letter are not significant at p<0.05; capital letter for different between plots and small letter for different between varieties)

<table>
<thead>
<tr>
<th>Plot</th>
<th>PCS (cm$^2$)</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>Plot (+)K</td>
<td>18.9 Aa</td>
<td>19.0 Ba</td>
</tr>
<tr>
<td>Plot (-)K</td>
<td>24.7 Aa</td>
<td>27.1 Aa</td>
</tr>
<tr>
<td>Mean</td>
<td>21.76 ab</td>
<td>23.0 a</td>
</tr>
</tbody>
</table>