Photosynthesis in Seedling, Juvenile and Adult of Oil Palm (*Elaeis guineensis*)

Kingkaew Apichatmeta

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of
Master of Science in Technology and Environmental Management
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Major Program	Technology and Environmental Management

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This is to certify that the work here submitted is the result of the candidate's own investigations. Due acknowledgement has been made of any assistance received.

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I hereby certify that this work has not been accepted in substance for any other degree, and is not being currently submitted in candidature for any degree.

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ABSTRACT

Oil palm (*Elaeis guineensis,* Jacq.) is recognized to be a highly important commercial crop in Thailand and improvements are needed in oil palm plantation management, but research information for development of oil palm plantation and increases in production yields needed. Photosynthesis potential on different age in oil palm plant has been studied using Pulse Amplitude Modulation (PAM). Adult oil palm has the highest photosynthetic potential, followed by juvenile and seedling plants. The photosynthesis potential is positively related with oil palm age, leaf surface area and chlorophyll content in the leaves. This reflects a marked difference in the morphology of adult leaves compared to juvenile/seedling leaves. The Oil palms required different optimum irradiance at different ages and the diurnal patterns of photosynthesis is different in adult and juvenile/seedling leaves: seedlings had optimum irradiance in the morning at 09:00 which contrasts with the adult, which has maximum photosynthesis in the afternoon at 15:00. Preliminary experiments on the short-time effects of additional nutrition have been conducted in seedling oil palm (N-sources, KCl and $CaHPO_4$). A negative effect on photosynthesis potential was demonstrated from this study, although some added nutrients had less effect than others on the photosynthesis potential of the seedling plant.

Keywords: *Oil palm, Photosynthesis, Optimum irradiance, Gross photosynthesis, PAM machine, Electron transport rate*

ชื่อวิทยานิพนธ์ ศักยภาพการสังเคราะห์แสงของปาล์มน้ำมันในเมล็ดงอก ต้นอ่อน และต้นเต็มวัย
ผู้เขียน นางสาวกิ่งแก้ว อภิชาติเมธา
สาขาวิชา เทคโนโลยีและการจัดการสิ่งแวดล้อม
ปีการศึกษา 2558

บทคัดย่อ

ปาล์มน้ำมัน (*Elaeis guineensis*, Jacq.) เป็นพืชเศรษฐกิจที่มีความสำคัญในลำดับแรก ๆ ของประเทศไทย แต่ยังขาดข้อมูลด้านต่าง ๆ ที่ช่วยสนับสนุนในการเพิ่มผลผลิต การศึกษาวิจัยศักยภาพของการสังเคราะห์แสงในแต่ละช่วงวัย ได้แก่ เมล็ดงอก ต้นอ่อน และต้นเต็มวัย จะเป็นข้อมูลซึ่งมีสำคัญต่อการพัฒนาศักยภาพของปาล์มน้ำมัน การศึกษาครั้งนี้ใช้วิธีการวัดการส่งผ่านอิเล็กตรอนด้วยเครื่อง PAM (Pulse Amplitude Modulation) พบว่าค่าศักยภาพการสังเคราะห์แสงโดยรวมสูงที่สุดในต้นเต็มวัย ต้นอ่อนและเมล็ดงอกตามลำดับและปัจจัยที่ตรวจวัด ได้แก่ ปัจจัยพื้นที่ผิวใบและปริมาณคลอโรฟิลล์ที่มีความสัมพันธ์กันในทิศทางเดียวกันกับช่วงอายุของใบปาล์มน้ำมัน รวมถึงความเข้มของแสงที่เหมาะสมในแต่ละช่วงวัยของเมล็ดงอกปาล์มน้ำมัน ซึ่งต้องการความเข้มแสงที่เหมาะสมในช่วงเช้า เวลา 09:00 น. ส่วนต้นเต็มวัยต้องการความเข้มแสงที่ เหมาะสมในช่วงบ่าย เวลา 15:00 น. ผลของการเพิ่มปริมาณสารอาหารที่สำคัญ เช่น ไนโตรเจน โพแทสเซียมคลอไรด์ และฟอสเฟต ในปริมาณที่เกินความต้องการของเมล็ดงอกในระยะเวลาสั้นซึ่งส่งผลให้ศักยภาพการสังเคราะห์แสงลดลงเมื่อเทียบกับเมล็ดงอกปาล์มที่อยู่ในสภาวะสิ่งแวดล้อมที่มีความปกติ ซึ่งสารอาหารแต่ละชนิดส่งผลต่อการส่งผ่านอิเล็กตรอน ผลผลิตของการสังเคราะห์แสงในระดับที่แตกต่างกัน แต่โดยรวมสารอาหารที่เพิ่มขึ้น ทำให้ศักยภาพการสังเคราะห์แสงของเมล็ดงอกปาล์มน้ำมันลดลง

คำสำคัญ: *ปาล์มน้ำมัน, การสังเคราะห์แสง, ความเข้มแสงที่เหมาะสม, การสังเคราะห์แสงทั้งหมด, เครื่อง PAM fluorescence, การส่งผ่านอิเล็กตรอน*

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CHAPTER 1

Introduction

1.1 Background and rationale

Oil Palm belongs to family Arecaceae and is an important plant for industrial oil production in daily life for example cooking oil, because it is rich in nutrition, containing fatty acids and vitamins and is used to make, instant cream, snacks, margarine, nutrition food etc. (Paiboon, 2007). Moreover, oil palm is the major component in cosmetic products and is used in machinery industries, etc. (Dufrene and Saugier, 1993). Governments of Thailand give strong support to develop oil palm industry as an important substitute energy as a liquid fuel (biofuel).Oil palm is one of the most important crop plants in Thailand because it is a highly productive plant in terms of production per area, low investment and effectively converts solar energy to liquid oil. Oil palms produce 3.75 – 5 ton/hectare/year compared to other oil-bearing plants and can provide a good income for farmers and other sectors (Malaysian Palm Oil Council, 2012).

At present, oil palm production in Thailand ranks fifth in world production. Oil palm plantations are widely planted in many provinces in the southern part of Thailand such as Krabi, Suratthani, Chumporn etc. (Kasikornthai Research Center, 2013). However, most of the oil palm oil produced is consumed in Thailand and the government plans to increase its production for export. The government encourages the expansion of the plantation area, and improvements in production are greatly needed.

The Oil palm industrial development plan during 2008 – 2012 aimed to expand oil palm plantation to 400,000 hectares and replacement of an old low production oil palm type with a higher production variety one over an area of 80,000 hectares, which will increase production from 18.75 ton/hectare to 21.875 ton/hectare (Agricultural Economic Office, 2010).

The government strategies for expanding oil palm plantations in order to increase their production for export inside ASEAN are related to aim of the ASEAN Free Trade Area (AFTA) for oil palm and seedling the market. These issues target the extension of

the ASEAN market and prepare for the projected ASEAN Economic Community: AEC which began in 2015 (Agricultural Economic Office, 2010). Even though oil palm has high economic potential and will strengthen food security and energy resources, oil palm production in Thailand is only 3% and ranked 3rd in the world production and investment is 4 times higher in Malaysia compared to in Thailand (Agricultural Economic Office, 2010).

Rather few research articles have been published in the open literature on developing and raising oil palm production, and improve return on investment. Not only that, but physiological knowledge of the Oil Palm is also poor, for example, photosynthesis capability of seedling, juvenile and adult, daily water requirement, response to fertilizers, ability to cope with water-logging and acid soils etc. This study aims to investigate the photosynthesis capability in different stage of oil palm from seedling to adult stage. The information will be greatly useful to set up suitable plantation area criteria for each stage of oil palm in the future. Oil palm is now the most important supplier of vegetable oil in the world but the oil it produces is ultimately a product of its photosynthesis which is poorly documented.

1.2 Review of related literature

1.2.1 Oil palm

Oil palm production in Thailand compared to other oil palm producer countries of 2010 – 2011 are shown in Table 1.1

Table 1.1 Oil palm harvested area, production and yield of major countries in the world

Country	Harvested area (1,000 rai)		Production (1,000 ton)		Yield per rai (Kg)	
Year	2010	2011	2010	2011	2010	2011
Indonesia	35,875	38,063	97,800	101,700	2,726	2,672
Malaysia	25,063	25,063	87,825	87,825	3,504	3,504
Thailand	3,552	3,747	8,223	10,777	2,315	2,876

Food and Agriculture Organization of the United Nations 1) Update by (Office of Agricultural Economics, 2013), 1 rai = 0.16 ha

Ecophysiology of Tropical Crops (Oil Palm)

The commercial oil palm (*Elaeis guineensis,* Jacq.) is a member of the subfamily Cocoideae (Verheye, 2002) originated in West Africa. The countries of origin of oil palm are the West and Central African coastal belt between Guinea and northern Angola. Oil palm is a member of a genus containing two main species: *E. guineensis* or African oil palm, and *E. melanococca* (Gaertn.) or American oil palm; the latter is only valuable for hybridization and does not produce useable amounts of vegetable oil. The genus Elaeis is a member of the family Arecaceac (Palmae) in subfamily Cocoideae (which also includes the coconut palm). Oil palm is a tropical tree crop which is mainly grown for its industrial production of vegetable oil. It is a typical estate crop, grown and harvested over large uniform areas with a centrally located processing factory.

For optimal growth and production, the crop requires a high and year round rainfall with little or no dry season and stable high temperatures; soils should be deep and well drained near enough to a central oil mill to allow rapid industrial handling after harvesting. Palm trees can also be observed in village gardens as a kitchen source of Palm Oil and edible seeds where they provide oil for local consumption at the village level, but in that case both yield and oil quality are much lower. Oil palm plantations need the clearance of large areas of land. They often require the expropriation of land and the cutting down of extensive forest areas. In Thailand, many former rubber plantations have been converted to Oil Palm and so the two plantation crops compete for the same land. At present Oil Palm offers better returns and so rubber plantations are being replaced by Oil Palm but in the future the situation might reverse and Oil Palm land might be converted back to rubber plantation.

Oil palm (*Elaeis guineensis* Jacq.) can be separated into 3 types (Figure 1.1) based on fruit type, which is controlled by a pair of shell thickness control-genes (Suratthani Oil Palm Research Center, 2010)

(1) **Dura**: shell thickness 2-8 mm, comprising 25-55% of weight of fruit, no fiber ring, medium mesocarp content of 35-55% by weight, but up to 65% in Deli Dura palm; less productive but hardy variety, kernels 4-20%, well adapted to non-commercial village gardens.

(2) **Pisifera**: shell-less, with small pea-like kernels (3-8%) in fertile fruits of little commercial value, because of its high abortion ratio, but important for cross breeding commercial palm, medium mesocarp 92-97%, with fiber ring.

(3) **Tenera** shell thickness 0.5-4 mm; comprising 1-32% of weight of fruit; medium to high mesocarp content of 60-90%, but occasionally as low as 55%; kernel 3-15%, this variety is the result of a hydridization of **Dura** and **Pisifera**, and has a high

commercial value. The Suratthani 2 variety used in the present study is a Tenera type *Elaeis guineensis* Jacq. var. Suratthani 2.

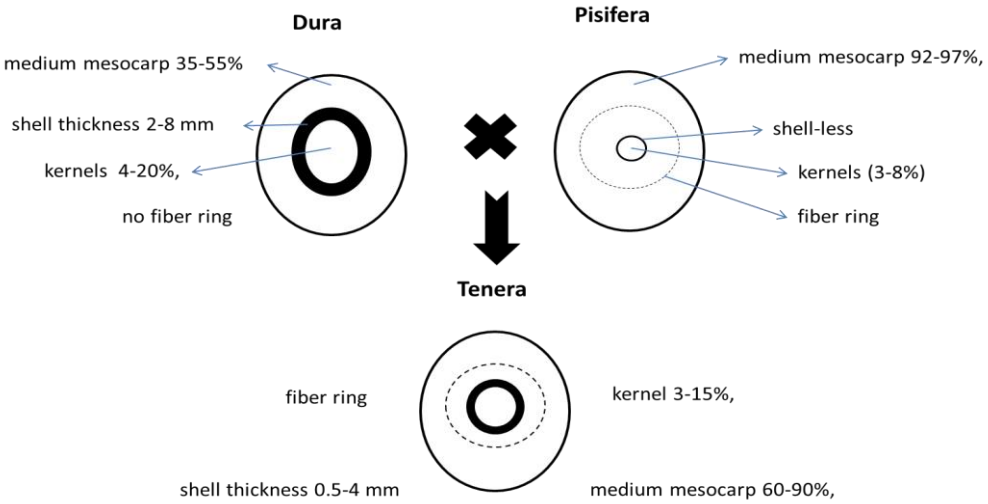

Figure 1.1 The seed of the oil palm

Oil palm as a rain-fed crop in Southern Thailand

Oil palms are successfully cultivated in areas of very heavy to moderate rainfall. In areas with very high rainfall (> 5000 mm), the rainfall is usually in excess of evapotranspiration. In such areas, constant cloudiness and water-logging could limit the productivity of Oil Palm plants (Hartley, 1977).

1.2.2 Typical C3 plants

Plant species, which use the Calvin-Benson cycle to form fixed carbon products, are called C3 plants (Klass, 1998; Atwell, *et al.*, 1999). This cycle produces the 3 carbon intermediate 3 phosphoglyceric acid and is common to trees, fruits, legumes, grains, and vegetables. C3 plants usually exhibit lower rates of photosynthesis at light saturation, sensitivity to oxygen concentration, rapid photorespiration, and high CO_2 compensation points when compared to plants with the other type of the photosynthetic system called C4 photosynthesis. The CO_2 compensation point is the CO_2 concentration in the surrounding environment under which more CO_2 is respired by the plant than is photosynthetically fixed and so net photosynthesis is zero. Typical C3 biomass species are alfalfa, barley, cotton and wheat and eukaryotic algae such as *Chlorella*. In general, C3 plants grow favorably in cooler climates (Klass, 2004). Plants with high light saturation points are called sun-plants and

typically have light saturation points higher than 500 µmol photons m^{-2} s^{-1} (or about ¼ of full sunlight). Plants with saturation points well below 500 µmol photons m^{-2} s^{-1} (200 µmol photons m^{-2} s^{-1} or lower) are called shade plants. Most plants are capable of some degree of photo adaptation and some plants change in light saturation characteristics as they grow, for example juveniles of many forest plants are shade-adapted but are typical sun plants as adults. That is why the juveniles of tree crops like oil Palm often need to be grown in a shade house before being moved to exposure to full sunlight.

1.2.3 Photosynthesis

Photosynthesis using Infrared Gas Analysis (IRGA)

CO_2 strongly absorbs infrared light and so the concentration of CO_2 in air can be measured by measuring the absorption of infrared light when a column of air is irradiated with infrared light. Infrared Gas Analysers monitor CO_2 in the air using a column of air in a small chamber irradiated with an infrared laser and the infrared light which passes through the air column that is not absorbed by the CO_2 present is measured using a photo-diode. IRGA machines are highly accurate, but very expensive and despite a great deal of research and development are still difficult to use in the field. A crucial disadvantage of IRGA is that data acquisition is very slow. Open IRGA systems are configured to allow air from a single source to enter both the analysis and reference lines of the IRGA. Air is continuously passed through the leaf chamber (to maintain CO_2 in at fixed concentration) and measurements of photosynthesis and the Transpiration are based on the differences in CO_2 and H_2O in the air stream that is the flowing into the leaf cuvette (reference cell) compared to the of air stream flowing out of it (sample cell). The rate of the CO_2 uptake is used to assess the rate of photosynthetic carbon assimilation. Many IRGA machines also monitor water vapour because water vapour also strongly absorbs photons in the infrared part of the spectrum. The rate water loss is used to assess the rate of transpiration (measured on a leaf area basis) (Valentine, *et al.*, 2013).

IRGA machines measure Net photosynthesis (Pn) as the rate of CO_2 taken up by leaves or the other plant material. An IRGA does not measure Gross Photosynthesis (Pg). Pg can be estimated if the respiration rate (production of CO_2) is also measured using the IRGA by measuring CO_2 production by the specimen in the dark. Thus to adequately measure photosynthesis in a plant both light and dark measurements of CO_2 flux between the plant and the air are necessary. IRGA methods give both respiration and Pn estimates from which Pg can be calculated.

Theory of Infrared Gas Analysis

Heteroatomic gas molecules absorb radiation at specific infrared (IR) wavebands, each gas having a characteristic absorption spectrum. Infrared gas analyzers (IRGAs) measure the reduction in transmission of IR wavebands caused by the presence of CO_2 between the radiation source and a detector. The reduction in transmission is a function of the concentration of CO_2. The only gas normally present in the air with an absorption spectrum overlapping that of CO_2 is water vapour. Since water vapour is usually present in the air at much higher concentrations than CO_2, this interference is significant, but may be overcome simply by drying the air or measuring H_2O concentration by another IRGA (Long and Bernacchi, 2003; Atwell, *et al.*, 1999).

Limitations of IRGA

The theory of infrared gas analysis (IRGA), as used in plant physiology, and its incorporation into portable open gas-exchange systems for the measurement of leaf and canopy photosynthetic and water vapour exchange has been described above. The measurement of the maximum quantum yield of CO_2 uptake and the construction and use of field systems for measuring respiration have been developed (Long, *et al*., 1996). These off the shelf portable systems provide real-time measurements of CO_2 uptake (A), transpiration (E), leaf conductance (gl), and the intercellular CO_2 mole fraction (Ci). The precision of measurement possible with these standardized machines has meant that custom-built field and laboratory systems have largely been replaced. In parallel with the development of portable gas exchange systems has been the development of further instrumentation that is greatly extending the ability to interpret the basis of change in CO_2 uptake *in vivo*.

Gas exchange measurements

Measuring gas exchange is the most commonly utilized technique at present for commercial and research purposes in order to measure photosynthesis of individual leaves, whole plants or plant canopy. Gas exchange measurements provide a direct measure of the net rate of photosynthetic carbon assimilation. The main advantages of gas exchange measurements are instantaneous, non-destructive and direct. CO_2 exchange systems use enclosure methods, where the leaf is enclosed in a transparent chamber. The rate of CO_2 fixed by the leaf is determined by measuring the change in the CO_2 concentration of the air flowing across the chamber. Because ambient atmospheric CO_2 concentration is only 0.04 % (400 ppm), it is difficult to measure photosynthetic CO_2 uptake and sensitive sensors are needed. Calibrated gas mixtures are needed for calibration (Long and Bernacchi, 2003).

Using an IRGA to measure photosynthesis of plants has inherent practical difficulties. It is very difficult to seal the plant chamber of an IRGA machine properly so there are no leaks. Leaks are a continuous problem. The geometry of illumination of the leaf chamber can present major difficulties in judging whether the measurements are realistic for plants growing in the field. Even the best designed IRGA machines are still difficult to use in actual field situations. The rate of data acquisition is very slow because measureable changes in the CO_2 concentration of the entire volume of the experimental system need to be made. In practice it takes about 30 minutes to 1 hour to obtain one estimate of net photosynthesis or respiration.

Limitations of photosynthetic measurements

The temperature optimum for photosynthesis is broad, then crop plants have adapted to a relatively wide range of thermal environments. The crop plants can adapt to the slow increase in temperature, global warming event may not be seriously affected to them. Leaf photosynthetic rates can be varying within or between species but is often not directly relate to productivity (Abrol and Ingram, 1996)

There is increasing temperature effect on rice based on leaf carbon dioxide assimilation (net photosynthesis) study, but the effect is continuing for temperature that not over than 41 °C. High variability in leaf CO_2 assimilation can be observed within rice genotype (Egeh, *et al.*, 1994). Observations such as the above indicate that of the gross photosynthesis compensates for temperature because respiration is very temperature sensitive and increases by a factor of about 2 for every increase in temperature of about 10 ^{o}C. Thus net photosynthesis would decrease sharply with temperature increase if gross photosynthesis did not increase to compensate for the increased respiration with increased temperature.

Consequently, any theory designed to investigate the connections among plant diversity, plants production, plant community stability, and the spatial distribution and supply rate of soil nutrients needs to incorporate an appreciation of the existence of these mechanisms. There is a growing number of ecological theories and plant models, in particular in the field of prairie grassland restoration, designed to investigate and predict relationships between plant diversity has substantially affected and plant community production that require for their implementation species specific information in plantation factor has maximum relative growth photosynthesis rate, root can be nutrient uptake rates, patterns of root biomass allocation, nutrient productivity. At least some sets of measurements that describe the spatial distribution patterns of roots, like root lateral spread, root depth, root length, and root surface area are also needed (Biondini and Grygiel, 1994).

Such models have not been applied to tropical plants to any significant extent. In particular, a great deal more basic measurements of photosynthesis under tropical conditions are needed based on the further development of modulated chlorophyll fluorometry (PAM), differential oxygen analysis and higher resolution infrared gas analysers suited for the measurement of non-steady state changes in CO_2 fluxes (Bloom, *et al*., 1980; Laisk, *et al*., 2002; Maxwell and Johnson, 2000).

The measurements photosynthesis using a variety of methods makes it possible to combine gas exchange and fluorescence information. Such considerations also highlight some of the pitfalls of the off-the-shelf of gas exchange systems (IRGAs). By contrast with the earlier custom-built of gas exchange systems, the modern commercially available systems enclose very small areas of the leaf, typically less than 10 cm^2 and often as small as 2 cm^2 (Long, *et al*., 1996). This has the advantage that, given the variability across of leaf surfaces, the measurements will be less prone to the errors in calculations resulting from the spatial heterogeneity of the stomata conductance and the photosynthetic capacity (Cheeseman, *et al*., 1991). It also has the advantage that the exact area is known, with the exception of the small or narrow leaves which do not fill even these small chambers. The downside is that a small area, by definition, will be having a larger edge-to-area ratio. In the older chambers typically used in the custom-built systems, the entire leaf or even a whole plant was enclosed and properly sealed. In the commercial chambers, only a portion of a leaf is sealed into the chamber. A seal is achieved with close fitting cell foam gaskets on both surfaces of the leaf isolating a small area. That is fine in theory but the practical reality is that it is extremely difficult to achieve an effective seal and impossible on some types of leaves, for example on a cactus. Much has been learned from the application of these systems in measuring the response of leave respiration to elevated (CO_2), but it has also been learnt how misleading the results from these systems can be if the attention is not given to potential errors (Long and Bernacchi, 2003).

Much of the problems with IRGA techniques revolve around the problem of gas leaks. Some the CO_2 can escape through the gasket of the photosynthesis chamber, this may not be a constant and will vary with the type of leaf and different leaves of the same plant species. Leaf CO_2 uptake (A) versus intercellular CO_2 concentration (Ci) of the curves may now be routinely obtained from commercial to gas exchange systems (Long and Bernacchi, 2003). Seal problems are worse among leaves with prominent veins where small air channels may form between the gasket and the sides of the vein. This is particularly significant at low CO_2 fluxes when to errors due to art factual apparent fluxes will have their greatest to effect and in the measurement of A/Ci responses (to determine the compensation

point of photosynthesis), when the differences between air outside and that within the chamber is greatest. A partial solution, recommended commonly by manufacturers is the measurement of the flux in the absence of the leaf. When the chamber is closed, a perfect of the seal should give a zero flux, regardless of the difference in (CO_2) between the inside and outside of the chamber. However, gaskets have some nonzero permeability and may be also release or absorb some CO_2 (Long and Haellgren, 1993). Estimates of leaks are used to correct the CO_2 fluxes. However, when the leaf is placed in the chamber additional leaks may be introduced. There are four partial solutions.

(1) Use a dead leaf, formed by rapidly the drying a live specimen and establish the rate of the leakage at each (CO_2) that will be used in the constructing of the A/Ci response.

(2) Enclosure of the chamber in a container filled with the gas mixture that is being introduced into the chamber. One means to achieve this is to supply in the outer container exhaust air from the system. Such setups are not commercially available and have to be purpose-built. Edge the effects occur because the gasket has a finite thickness and the gasket and any other wall the structure above the leaf will to affect radiation in the chamber, unless the light source is a parallel beam at 90° of the leaf surface. In the field if natural a sunlight is used as the light source, from the lower the sun angle the greater this shading the effect may be. This problem is alleviated if an artificial the light source is placed above the chamber. The gasket will also cause the photosynthesizing of surface to be surrounded by tissue in the darkness that is of respiring. This respired of CO_2 will decrease the measured net flux (Long and Bernacchi, 2003).

(3) The last and most obvious problem with gas exchange experiments is the problem of wounding effects. It can be very inconvenient to attempt to measure gas exchange of a leaf *in situ*. It is tempting to cut a disc or a square of leaf and place it in the chamber of an IRGA but if a leaf is removed from a plant for an IRGA experiment then wounding effects might be expected. This is exacerbated by the long incubation times (usually 30-60 minutes) needed using IRGA methods compared to the PAM techniques (usually only 2-3 minutes are needed after a darkness adaptation period of about 10 minutes). Unfortunately, wounding effects vary enormously in the plant leaves. Leaves of some plants remain fully functional for hours after cutting and others shut down in a few minutes. This problem has to be investigated on the specific species being used for a project. For example, other students in the laboratory have found that mangroves leaves shut down very quickly after being removed from a plant. Oil Palm leaves were found to shut down slower than mangroves but

nevertheless wounding effects were observed and so removal of leaves from a plant was avoided in the present study using PAM techniques.

(4) Even when an IRGA is used properly the methods still has a major inherent limitation: it is very slow because net fluxes have to be measured over time periods long enough to be measureable. In practice this means that it takes about 30 minutes to 1 hour to obtain a valid measurement of the Pn or respiration. The longer a plant leaf is incubated in the chamber of an IRGA the more likely there will be experimental artifacts and the amount of data obtainable is very small compared to the data acquisition rate of a PAM machine.

Optical activity and chlorophyll content in leaves of intact plants

The basis of chlorophyll fluorescence measurements

The principle underlying in the chlorophyll fluorescence analysis is relatively straightforward in leaves. Light energy absorbed by chlorophyll molecules in a leaf can undergo one of three fates: it can be used to drive in photosynthesis (photochemistry), excess energy can be dissipated as heat or it can be re-emitted as the fluorescent light from the chlorophyll in PSII. These three processes occur in competition, such that any increase in the efficiency of one will result had decreased in the yield of the other two. Hence, by measuring the yield of chlorophyll fluorescence, information about changes in the efficiency of photochemistry and heat dissipation can be gained basically by subtraction and using the thermodynamics first law (Maxwell and Johnson, 2000).

Chlorophyll Fluorescence

The chlorophyll fluorescence can be measured using a Handy PEA portable fluorescence spectrometer (Percival and Fraser, 2002) and can also be directly measured using the Junior PAM. Before the operation, leaves have to placing in darkness for 30 minutes by attaching light-exclusion clips to the leaf surface area of whole trees (Percival and Fraser, 2002). Fluorescence values recorded by the WinControl Software of the PAM were as follows:

(1) Ratio of the variable fluorescence to the high fluorescence, or Fo (Minimum of fluorescence), as a measure of the stability of the light-harvesting complex (Yamada, *et al*., 1996).

(2) The ratio of variable fluorescence (Fv = Fm – Fo) to the maximum (Fm) fluorescence (Fv/Fm, which represents in the maximum quantum yield of Photosystem II), changes in response to high light and chilling in temperatures, which in turn are highly

correlated with the quantum yield of the net photosynthesis (Demmig and Björkman, 1987; Bolhar-Nordenkampf, *et al*., 1989; Adams, *et al*., 1995).

Chlorophyll fluorescence - modulated and unmodulated

Chlorophyll fluorescence is a rapid, sensitive and reliable method for estimating the activity of the light reactions of photosynthesis (Schreiber, *et al*., 1986; Genty, *et al*., 1989; van Oorschot and van Leeuven, 1992; Maxwell and Johnson, 2000). Despite the fact that total fluorescence is small (only 3% of absorbed light when PSII is closed), its measurement is quick and easy, and it is intensity of the flash is inversely proportional to the efficiency of photosynthesis rate of plants (Krause and Weis, 1991). In the following minutes the level of fluorescence decreases, which is known as fluorescence quenching (Schreiber, *et al*., 1986; Demmig and Björkman, 1987). A part of the energy absorbed by chlorophyll in the PSII can be used in photochemical reactions ($Fv=Fm-F_0$; Gilmore, 1997). Photochemical efficiency of photosynthesis (quantum yield, Y) is calculated from the ratio of Fv/ Fm (Schreiber, *et al*., 1986; Genty, *et al*., 1989). The optimum value of fluorescence for a healthy level of C3 plants was around 0.8 (Björkman and Demmig, 1987), so this parameter can be used as an indicator of whether plants have been exposed to stress factors (drought, water flooding, herbicides, etc.), i.e. whether the inhibition of photosynthesis has taken place.

Responses to drought

Several factors will promote more frequent droughts in the future because of climatic change such as higher temperatures and higher variability in precipitation and shifting climatic patterns. The higher temperatures in addition also increase the vapor pressure deficit in plants if evapotranspiration does not also increase. Greater variability in precipitation has two implications for plant water balance: longer periods without water, and less captured in the soil in the more intense storms (Ryan, 2011). In general, photosynthesis in land plants is limited by water, not irradiance or CO_2 (Atwell, *et al.,* 1999). Many C3 plants close their stomates during the middle of the day to prevent excessive water loss hence limiting availability of atmospheric CO_2. This phenomenon is called midday inhibition. Oil Palm does not typically shed leaves, but drought also ‘weakens’ trees and makes them more the susceptible to insect attacks and the pathogens (McDowell, *et al*., 2008). Under such stress Oil Palm plants will close their stomates over much of the day hence severely limiting fixation of CO_2. The plants Growth rate can be reduced through impairment of cell division and cell expansion (physical of plants force needed to sustain enlargement) (Hsiao, 1973). Such effects are apparent at a lower water stress threshold than photosynthetic inhibition

(Hsiao, *et al.*, 1976). In fact, a variety of physiological processes respond at different thresholds of plant water potentials (Hsiao, 1973; Ditmarová, *et al*., 2010), so that the severity of the drought season will influence the physiological response. Drought responses also vary with the ecology of the plant. Species adapted to low precipitation climates can survive low soil, water potentials that would kill or seriously wound trees in more moderate climates and trees in moderate climates might suffer from 'drought' that would be normal for trees in xeric (arid) climates. Finally, we know that there are many mechanisms of drought tolerance, and that many of these involve coordination within the whole tree (Atwell, *et al.*, 1999). Typical locations where the oil Palm is grown have a distinct wet and dry season and it would be expected that the plants respond differently to water stress in different times of the year that are normally the dry season compared to during the wet season.

Net photosynthesis and respiration

At the same time as leaves absorb the CO_2 from the atmosphere during photosynthesis, they consume O_2 and release CO_2 from respiration. Respiration is the process of photosynthesis. It is the process by which organic compounds are oxidized to produce the energy needed to maintain plant functions and grow new plant tissues. For glucose, the overall chemical reaction is:

$$C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O$$

As in the case of photosynthesis the actual reaction is closer to

$$C_6H_{12}O_6 + 6\ H_2O + 6\ O_2 \rightarrow 6\ CO_2 + 12\ H_2O$$

because water is both a substrate and a product of respiration.

The respiration rate can be depends on the biochemical quality of the plant apparatus and increases exponentially with value of temperatures in the environment. This respiration is different from photorespiration, which is driven by a fixation of oxygen rather than CO_2 by RUBISCO, and occurs simultaneously with photosynthesis in leaf cells (Atwell, *et al.*, 1999). The CO_2 uptake by the plant had difference between during photosynthesis and quantity of CO_2 loss during on leaf respiration is the net CO_2 uptake by a leaf during photosynthesis. Hence, the CO_2 evolving in the background when a leaf is photosynthesizing in the light is not easy to quantify because it is the sum of oxygen consumption by oxidative photophosphorylation as well as photorespiration. Gross photosynthesis is therefore not easy

to estimate from IRGA data. Physiologically based, large-scale models in mixed species forests have typically used lumped-parameter approaches (e.g. Corley, 1973; Corley, *et al*., 1971), where for example, the photosynthetic rate of the entire forest canopy is represented by a single equation. This is obviously unrealistic. Although direct physiological of plant measurements in adults broad of leaved deciduous from forests are limited for this studies because canopy access is difficult, so studies have established within crown variation leaves in environmental factors of photosynthesis and their effects on photosynthesis rate (Aubuchon, *et al.*, 1978; Caldwell, *et al.*, 1986), relationships of annual ontogeny to the net carbon fixation (Dougherty, *et al*., 1979), and the physiology of plant during water deficits (Weber and Gates, 1990). Such work is relevant to estimating photosynthesis in Oil Palm but there is a lack of similar work on tropical trees and models developed for temperate trees might not necessarily apply to tropical trees.

Light-Dependent Reactions

In the Light-Dependent Reactions, the first process happens in the thylakoids of the chloroplasts in the leaves and are the "light - dependent" reactions occur in The photosystems I and II had absorb the photons from the sunlight (light source) and process them through the membranes of the thylakoids in chloroplasts simultaneously. The photons had excite electrons (Electron transport rate) in the chlorophyll which then move through the electron transport chain and causes NADP to combine with H^+ forming NADPH. At the same time, ADP (adenosine diphosphate) has come from the dark reaction and a third phosphate chain is bonded forming ATP (adenosine triphosphate) to feed the Calvin Cycle next. ATP is the important source of all cellular energy (Griffin, 1998) and so ATP formed by the light reactions of photosynthesis can also be used for other processes apart from carbon fixation by the Calvin Cycle. In terms of what a PAM machine measures, the PAM machine measures electron flow through PSII and not through PSI.

Dark Reactions

Dark reactions are also known as the Calvin Cycle, the Calvin-Benson cycle, and light-independent reactions of photosynthesis. The point is that they do not require sunlight to complete their processes. After ATP and $NADPH_2$ are formed in the light reactions of photosynthesis, CO_2 is transformed into carbohydrate using ATP and $NADH_2$. This happens during the Calvin Cycle in the stroma. ATP and NADPH are used to combine CO_2 and water to make the end product of glucose. The ADP and $NADPH+H^+$ are recycled to the light-dependent side of photosynthesis to start the process over (Griffin, 1998).

1.2.4 PAM (Pulse Amplitude Modulation) Fluorometry

In the 1960s, more and more people used chlorophyll fluorescence to study the photosynthesis and plant photosynthetic behavior. One of the first commercially available instruments was the PAM 100 from Walz, developed by Ulrich Schreiber about 1980. The PAM machines are pulse amplitude modulated fluorometers which means, that they measure the fluorescence yield at saturating light intensities as well as in a very low background state which can be considered equivalent to a zero light fluorescence measurement in darkness. In addition the introduction of the saturating pulse method allows measurement of the Quantum Yield the percentage of light actually used and the (relative) Electron Transport Rate (rETR). If the absorptance of the plant material is known (Ritchie and Runcie, 2014) it is possible to convert rETR into ETR. The absorptance of Oil Palm has been measured experimentally in the present study and confirmed measurements made in a previous study which were part of the testing of a machine developed to routinely measure the absorptance properties of leaves (Ritchie and Runcie, 2014).

A junior PAM was used in the present study. The junior PAM is in the tradition of the PAM 100. Junior PAM is the low end for "Standard PAMs" (e.g. Mini PAM, Microscopy PAM, Micro Fiber PAM, Water PAM, Aqua PAM) (Gademann Instruments GmbH, Würzburg, Germany). All the basic applications work automatically: Actinic + Yield, Light Curve, Induction Curve and Recovery. Junior PAM comes with the same WinControl program, which controls the other instrument of the PAM family. Therefore, measures the same parameters as the more expensive PAM machines. The programming language of WinControl allows more complex and interactive routines. Unfortunately, the output data of a PAM machine has to be processed considerably to make it into a source of usable information (White and Critchley, 1999; Rascher, *et al.*, 2000; Baker, 2008; Ritchie, 2008, Ritchie and Bunthawin, 2010a, 2010b; Ritchie, 2012). Documentation of Walz PAM machines is written in difficult-to-understand German, English and the WinControl software is not very user-friendly.

The data output of the WinControl software can be analysed in detail using methods described by Ritchie (2008); Ritchie and Bunthawin (2010a, 2010b) and Ritchie (2012, 2014) using non-linear least squares fitting methods. Dose-response curves for inhibitory compounds such as chlorine on the green alga, *Chlorella*, have been successful (Saetae, *et al*., 2013) and for arsenic (Ritchie and Mekjinda, 2014). ETR is normally measured as mol (e^-) m^{-2} s^{-1} and can be related to oxygen evolution ($4e^- = 1\ O_2 = 1\ CO_2$ based on the basic overall reaction of the light reactions of photosynthesis $2H_2O \rightarrow 4e^- + 4H^+ + O_2$) and is a measure of Gross Photosynthesis (Pg). Hence, PAM machines directly measure Pg

but IRGA machines measure net photosynthesis (Pn). If the chlorophyll *a* content of leaves is known on a surface area basis ETR can be calculated as mol (e^-) mg Chl a^{-1} h^{-1} or mol (O_2) mg Chl a^{-1} h^{-1} (the conventional units used in photosynthetic studies). It is critical to understand that PAM machines measure Gross Photosynthesis not Net Photosynthesis because the PAM technique provides no the information on respiration. To estimate net photosynthesis (Pn) and relate it to growth, need information on respiration. On the other hand, if an IRGA is being used the machine gives information on respiration and the net photosynthesis (Pn) but not gross photosynthesis.

PAM has been successfully used to measure photosynthesis in Orchids, Pineapples, Water Lilies and Lichens (Ritchie and Bunthawin, 2010a, 2010b; Ritchie, 2012, 2014). Preliminary work on oil Palm seedlings and adult plants in the field were successful (Apichatmeta and Ritchie, 2016) and a manuscript has been submitted to Tropical Plant Biology (Apichatmeta, *et al.*, 2016). The standard experimental protocol for estimating photosynthesis in plants using a PAM is the Rapid Light Curve (White and Critchley, 1999; Ritchie and Bunthawin, 2010a, 2010b; Ritchie, 2010; Ritchie, 2012).

What PAM Fluorometry <u>Cannot</u> Do!

PAM machines are much too easily treated as a magic box. PAM machines can be very impressive but many users do not understand how to use them properly. It is important to realize that some published work is unreliable due to a lack of understanding of how the PAM machine actually works and the proper protocols to be followed.

Some difficulties with PAM machines

- PAM machines measure fluorescence, in other words the light that is <u>not used</u> for photosynthesis but is <u>re-emitted</u> as fluorescent light. Hence, Gross photosynthesis is calculated by subtraction of fluorescent photons from the visible light photons fired at the plant in a flash of light. The fact that photochemistry is calculated <u>*by subtraction*</u> means that on <u>some organisms</u> under <u>some conditions</u> PAM will give spurious results because the assumptions inherent in the calculation have been violated.

- Quantitative estimation of Gas Exchange from PAM data can be difficult. Need oxygen electrode, IRGA or ^{14}C data estimate correlation.

- Cannot measure enzymes such as RUBISCO activity.

- NADPH & ATP levels can be inferred from non-photochemical quenching data but not measured directly.

- PAM cannot provide any information on respiration. This is a major limitation of PAM machines in studies of physiological stress in plants.

- Cannot measure net photosynthesis and so cannot measure the growth of plants, but this is possible if respiration data is available from other sources.

Effects of high light saturation on PAM Parameters

Three components of non-photochemical Chlorophyll fluorescence (NPQ) quenching can be the differentiated, as they possess differential relaxation times. The fastest relaxing Component qE (2–4 minutes) is related to the development of the pH-gradient ΔpH in the thylakoid lumen. As it the shows up at high irradiance conditions above the light saturation point of photosynthetic CO_2 assimilation, it has also been termed "high energy Quenching" coefficient qE, qT as the medium fast relaxing component (ca. 10–20 minutes) describes the "state transitions" of the two photosystems, whereas the slow relaxing Component qI (> 40 minutes) indicates the degree of photoinhibition of PSII. Lichtenthaler and Burkart (1999) measured the Chl fluorescence kinetics of clover (*Trifolium*) simultaneously with the CO_2-assimilation rates by placing the glass fiber fluorescence detection arm of the PAM fluorometer on top of the gas exchange cuvette containing the attached trifoliate leaflets of the clover plants. In rapid light curve protocols (White and Critchley, 1999; Ritchie and Bunthawin, 2010a, 2010b; Ritchie, 2012, 2014), two measurements of non-photochemical quenching are calculated by the Walz WinControl software, designated qN and NPQ. These are calculated using slightly different formulae based on different assumptions about minimal fluorescence (Ritchie and Bunthawin, 2010a, 2010b; Ritchie, 2012, 2014). Valid estimates of qN are always less than 1; calculated NPQ can vary from about 1 to up to 4 in some vascular plants. In vascular plants qN < NPQ but this is not necessarily the case in algae.

1.2.5 RAT (Reflectance Absorptance Transmittance) Measurements

The RAT uses a red-green-blue (RGB) LED diode light source to measure absorptances at wavelengths suitable for use with PAM fluorometers and infrared gas analysers (Ritchie and Runcie, 2014). The photosynthetic electron transport rate calculated from PAM data requires an estimate of how much light was absorbed by a leaf (Absorptance, Abt). In the Walz software a default value (Abt_F) of 0.84 is used, derived mainly from data using white light (Björkman and Demmig, 1987). Actual experimental measurements of absorptance on blue light (many PAM machines used a blue diode ≈ 465 nm) as the light source are typically much higher than the default value (Ritchie and Runcie, 2014).

Growth rates and Net Photosynthesis

The plant can be growth increment allows trees to respond by the changing environment such as rain season, dry season etc. The ability of the plant to resist strong wind and major losses or decrease of woody materials in tree, so while the remaining alive and erect, it is a direct consequence of the diameter in woody growth each year. The amount of woody had increment the produced each year is dependent upon of the proper functioning during alive and the photosynthesis productivity of the leaves. The food substances had ultimately generated by photosynthesis processes and the metabolic processes in the leaves (photosynthesis) will directly determine the amount of material in plant can be available for generating annual the increments. The annual increment of growth of a tree is a result of crown tree production which is a direct result of annual increment, transport efficiency and biomass volume. The incremental growth also mechanically supports the crown against dynamic forces of gravity, winds, precipitation and the tree own size, shape and the mass. Because the crown of the tree is provided with the raw materials and growth substances collected and the generated from the root, and the roots are provided with the food and growth substances generated from the crown, the physical distance and the biological health between living crown and absorbing root is critical. So those cells of plants between leaf and root must be stored, accumulate, defend, support, protect, electron transport, prevent waste, and conserve resources needed for keeping plants alive (Kim, *et al*., 2004). Oil Palm is a monocot and so technically does not produce wood but in functional terms oil Palm is a tree with leaves concentrated in a crown.

Photosynthesis and Nutrients

Some simple nutrient studies were part of the project but because of time restrictions such studies were restricted to seedling. Seedlings were readily available and could be grown in pots of sand and fed standard NPK-type fertilizers. A seedling became a juvenile when it had produced at least one new leaf and no longer had simply one cotyledon leaf. A laboratory version of a standard NPK fertilizer was used where manipulations of nutrient supply were required. Simple nutrient experiments on photosynthetic responses were be done using a PAM machine to monitor short term (≈ 3 hours) responses to added nutrients. In general, photosynthetic responses to nitrogen sources (ammonia, urea and nitrate) are reportedly easily detectable and are very rapid (this generalization was not borne out in the present study). Phosphate effects are generally difficult to detect using a PAM machine. PAM is known to be a sensitive detector of iron (Fe) deficiency because Fe deficiency has

direct effects on photosynthesis. Since many soils in Thailand are leached acidic lateritic soils experiments on aluminium toxicity in Oil Palm also appeared to be feasible.

1.2.6 Nutrient Effect Measurements

P (Phosphorus)

Phosphorus is one of the essential but micronutrient elements, plants do not require it in as large amounts compared to fixed nitrogen or potassium (Murphy and Riley, 1962). It is critical for photosynthesis and respiration process in plants because of its role in energy metabolism (NAD, NADH, ADP and ATP) and is also a critical component of RNA and DNA (Atwell, *et al.,* 1999). In conditions with insufficient phosphorus, plants are not able to convert the sugar to energy or fix carbon in photosynthesis (Spectrum Analytic, 2010; Ninnon, *et al*., 2010).

K (Potassium)

Even though potassium is not in plant compounds, it plays an important role on metabolic activities and physiological functions (Atwell, *et al.,* 1999). Potassium is essential for the function of the cytoplasm of the cells of plants. Imbalances between potassium and nitrogen ratio affects growth, plants will also be affected because nitrogen production by nitrogen-fixation bacteria also requires potassium (Spectrum Analytic, 2010; Ninnon, *et al*., 2010; Kant, *et al*., 2005)

N (Nitrogen)

Nitrogen (N_2) is not used directly by eukaryotic vascular plants. They use nitrogen compounds (fixed nitrogen) as N-sources: ammonia (NH_3^+, NH_4^+), nitrite, nitrate and urea are typical N-sources for plants (Atwell, *et al.,* 1999). Nitrogen is one of the important elements in structures and plant compounds. Nitrogen is involved in protein, chlorophyll and nucleic acids in plant cells. Unbalanced nitrogen in the plant has highly detrimental effects on the protein level, growth, and plant yields. The photosynthesis capacity of the leaf is significantly related to nitrogen contents; however, variation in photosynthesis capacity can be found between different species (Evans, 1989). Nitrogen ratio is significantly related to other minor nutrient such as potassium as in mention above on potassium subject, if improperly ratio between nitrogen and other nutrients, plants will suffer or harm not only their structure but also ability to recover from stress (Spectrum Analytic, 2010; Ninnon, *et al*., 2010). Nitrogen status also has important environmental consequences and so correct levels of fertilizer are needed (Hewitt, *et al.,* 2009).

NH_4Cl (Ammonium Chloride)

Ammonia is the simplest form of fixed nitrogen and is readily useable by plants but it may also have undesirable side effects: at high concentrations, particularly under alkaline conditions it is highly toxic (Atwell, *et al.*, 1999). Another undesirable side effect is that NH_4^+ tends to mobilize heavy metals. Given that soil NH_4^+ and Cl^- increased the uptake of heavy metals like cadmium (Cd) by root plants, it is expected that fertilizers containing ammonium chloride (NH_4Cl) influence Cd concentrations in wheat. Ohtani, *et al*. (2007) conducted a pot experiment and showed that are NH_4Cl fertilizer significantly increases Cd concentrations in the shoots of every plant compared with urea or ammonium sulfate fertilizers. It has been shown that NH_4Cl fertilizer had significantly increased the Cd concentrations in the rice and spinach shoots grown in pots compared with other nitrogen fertilizers, with such as the ammonium sulfate, ammonium nitrate, and ammonium dihydrogen phosphate (Ishikawa, *et al*., 2015).

$NaNO_3$ (Sodium Nitrate)

The Sodium nitrate ($NaNO_3$) contains nitrogen which is very important in the growth of plants. Plants take up nitrate then convert it to nitrite before finally to ammonia before incorporating it into organic compounds (Schuman, *et al*., 1973). Oversupply of nitrate can delay the production of fruit and flowers and too little can lead to stunted growth of plants and yellowing of leaves. The nitrogen (N) from sodium nitrate fertilizers is immediately available to plant roots (Trivedi, *et al*., 2015).

The Sodium nitrate ($NaNO_3$) has been used in industry in a large number of fields ranging from agriculture of plants to use in the food industry. Sodium nitrate ($NaNO_3$) is typically used to make fertilizers. As one of NPK fertilizer's main ingredients, Sodium nitrate ($NaNO_3$) acts as the substance that increases the amount of nitrogen (N) contained in the soil for plants (Atwell, *et al.*, 1999). The amount of fixed nitrogen (N) in the soil is crucial for plant growth since it helps the roots of plants used to growth thicker and are stronger as to the carbon production is increased (Nelson and Sommers, 1980). Other than Sodium nitrate ($NaNO_3$), standard NPK fertilizer also contains 2 other main ingredients which are potassium (K) and phosphorus (P). The Sodium nitrate ($NaNO_3$) component of NPK has a number of beneficial qualities such as being hygroscopic, it is easy to spread, and fairly steady consistency during storage. Both C3 and C4 plants (sugarcane, barley, beets, root vegetables and wheat) grow best with fertilizers containing Sodium nitrate ($NaNO_3$).

KCl (Potassium Chloride)

Both K and Cl are the main ions involved in the neutralization of charges on clay particles and organic matter in soils and as the most important inorganic osmotically active substances in plant cells and the plant tissues (Clarkson and Hanson, 1980; Atwell, *et al.*, 1999). K^+ and Cl^- are critical in the control of the opening and closing of stomates in leaves but this was demonstrated quantitatively only in 1996 (Talbott and Zeiger, 1996). The mechanisms for K and Cl uptake by plants have been topics of much research in plant physiology. The generally accepted model of high and low affinity sites for K uptake was presented by Epstein in the early 1960s but this has been replaced recently by the demonstration of specific K channels in the various cell membranes (Anderson, *et al*., 1992; Sentenac, *et al*., 1992; Atwell, *et al.*, 1999). The existence of Cl channels in plants has also been demonstrated (Lew, 1991; Lurin, *et al*., 1996).

$CaHPO_4$ (Superphosphate)

Superphosphates are generally used as the experimental standard, but there are many different phosphate structures found in soils such as pyrrophosphates and polyphosphates of varying availability to plants (Trenkel, 1997). Most phosphates are insoluble. The variability existence in the composition of phosphate sources can result in equivocated conclusions in studies with fertilizers (Chien, *et al*., 2010). Total phosphate in soils can be measured using standard methods (APHA, 1998) but it can be very difficult to estimate how much of it is actually available to plants. The ability of plants to mobilize insoluble forms of phosphate varies greatly for example plants such as oil Palm which typically grow on highly leached tropical soils are usually very good at mobilizing insoluble forms of phosphate (Atwell, *et al.*, 1999).

1.3 Objectives

1.3.1 To measure the photosynthesis rate of oil Palm each growth stage

1.3.2 To study some short-term nutrient effects on oil Palm photosynthesis to investigate the feasibility of using PAM to monitor the nutrient status of seedling oil Palm.

1.4 Scope

1.4.1 Study Photosynthesis in Seedling, Juvenile and Adult of Oil Palm (*Elaeis guineensis*) of Oil Palm by the junior PAM (Pulse Amplitude Modulation).

1.4.2 Study short-term nutrient (NH_4^+, NO_3^-, Na^+, K^+, P) effects on oil Palm's photosynthesis.

1.5 Expected outcomes

1.5.1 To improve cultivation of plants on oil Palm plantations in seedling, juvenile and adult stages by developing simple and rapid methods for estimating photosynthesis of Oil Palm plants using PAM technology which has not previously been used to full advantage (Suresh, *et al.*, 2010).

CHAPTER 2

Research Methodology

This chapter presents the methodology use in this project on Photosynthesis in oil Palm (*Elaeis guineensis*, Jacq., var. Suratthani 2). The overall process can be categorized into 3 studies or phases, starting with the conceptualization of the Photosynthetic rate in oil Palm and later measurement of the effects of absorption of nutrients on oil Palm photosynthesis.

Figure 2.1 Ripening oil palm fruits (a) oil palm seeds without the mesocarp (b) used for the experiment (from C. Sudsiri.)

2.1 Research design for Study Photosynthesis in Seedling, Juvenile and Adult of Oil Palm

Sampling of adult oil palm leaves were as follows:

Sampling oil palm leaves with no disease symptom, no deficiency nutrient appearances such as yellow leaves, deformed leaves and color leaves etc.

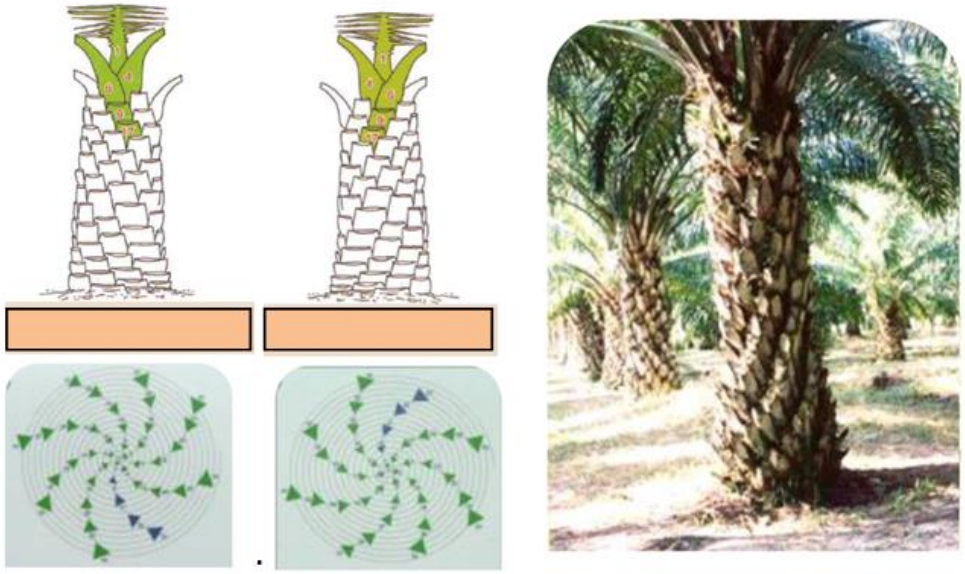

Figure 2.2 The trunk of the oil palm for adult plants (from Ninnon, *et al.*, 2010)

In adult oil palm plant leaf sampling, 17th pinnate leaf were selected and picked up the leaflet at the middle of the 17th pinnate leaf. The middle of the leaflet can be observed by foliar change from flatted form to triangular form (Ninnon, *et al*., 2010).

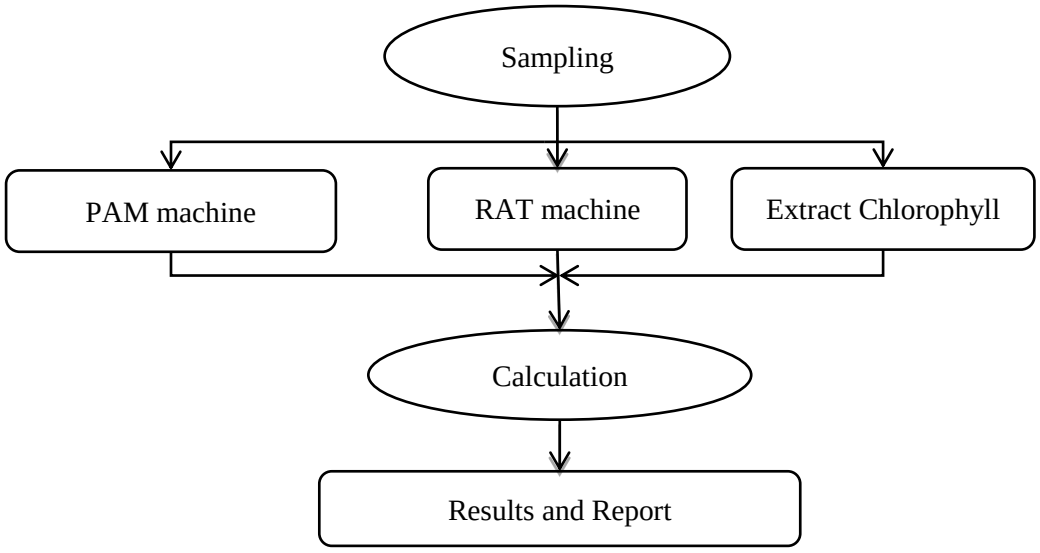

Figure 2.3 The research process

2.1.1 Material and Equipment

Measurement Photosynthesis by PAM machine

Figure 2.4 Seedling of oil palm plant still with seedling leaves

Figure 2.5 Juvenile in oil palm plant with leaves of adult morphology

Figure 2.6 Young Adult of oil palm plant. Note fruits and leaf bases.

The Oil Palm variety used for the study was *Elaeis guineensis*, Jacq. var. Suratthani 2. Selected leaves for seedling were: (1 year) 3–5 leaves palm (first bifurcate leaf-pinnated leaf) from Prince of Songkla University, Suratthani campus (Figure 2.1), juveniles: 3-5 years 30-70% unfolded leaf (Figure 2.3). Adult tree: (> 5 years) leaves 2-3 m long and pinnated leaf (Figure 2.4). The leaves of adult to be used for rapid light curves using the PAM machine had to be used soon after cutting (<15 minutes) because they rapidly lost turgor and rETR dropped dramatically. For seedling and juvenile plants it was practical to do PAM measurements of leaves that had not been removed from a plant. Measurements were routinely made during the middle of the day after dark adapting the leaves for at least 10 minutes following standard protocols for rapid light curve measurements described by Ritchie and Bunthawin, 2010a, 2010b and Ritchie, 2012. For diurnal curve experiments rapid light curves were performed on plants at 3 hour intervals from 6 a.m. to 6 p.m. (Solar Time) as follows: 6, 9, 12 a.m., 3 and 6 p.m. The light intensity at the study site showed a maximum of 2200 µmol m^{-2} s^{-1} during the year for Phuket, Thailand. As seen from the measurements during the various months irradiance was never limiting at the site. The atmospheric temperature at the Phuket had the site ranged between 34-37 °C.

Extraction of Chlorophyll

A small hole punch (9.7-mm diameter) was used to collect 73.9×10^{-6} m^2 of leaf tissue. Chlorophyll was extracted in Mg carbonate-neutralized ethanol. Incubation of the leaf disks in alcohol for a brief period (5 minutes) at 65°C was required for effective extraction of the chlorophyll. The heat treatment destroyed any chlorophyll are present and so the extracts could be stored on -20°C until they were assayed. Chl *a* and *b* was determined spectroscopically using a Shimadzu UV-1601 spectrophotometer using the equations of Ritchie (2006). Chl *a* was calculated as mg Chl $a\ m^{-2}$ of the projected leaf surface area, mg Chl $a\ g^{-1}$ FW, the Chl *b*/Chl *a* and Chl *a*/Chl *b* ratios were also calculated.

2.2 Research design for study of some short-term nutrient effects on Oil Palm photosynthesis

2.2.1 Nutrient Uptake/feeding experiments

Effects of added nutrients on seedling plants (1 year) were measured using rapid light curves performed on potted plants about 3 hours after the plants were watered with an experimental nutrient solution. It was important to infuse the plants properly with the experimental nutrient. Oil palm leaves were selected with no disease symptoms, no obvious nutrient deficiency symptoms and the standard nutrient addition was 10 % of concentrate (1 mol m^{-3}) because critical nutrient range is defined as the nutrient concentration at which a 10% loss of plant growth occurs (Fageria, 2009) (protocol in Figure 2.2). Leaves were kept in black cloth bags about 10 minutes before performing the rapid light curves, based on 16 leaves for each treatment.

Control

NH_4Cl (Ammonium Chloride)

$NaNO_3$ (Sodium Nitrate)

KCl (Potassium Chloride)

$CaHPO_4$ (Superphosphate)

2.3 Research tools

2.3.1 The collection of field data

Measurement of Photosynthesis using Pulse Amplitude Modulation (PAM) Fluorometry. PAM measurements were made a Junior PAM portable chlorophyll fluorometer (Gademann Instruments GmbH, Würzburg, Germany) fitted with a 1.5 mm diameter optical fiber and a blue diode (465 ± 40 nm) light source. PAM parameters (Y, rETR, qN, NPQ) were automatically calculated using the WinControl software (v2.08 & v2.13; Heinz Walz Gmbh, Effeltrich, Germany) as defined by Genty, *et al*. (1989) van Kooten and Snel (1990) and Krause and Weis (1991), using the standard default settings for rapid light curves (default absorptance factor, Abt_F = 0.84, PSI/PSII allocation factor = 0.5) to calculate the relative Electron Transport Rate (rETR) (Schreiber, *et al*., 1995; White and Critchley, 1999; Rascher, *et al*., 2000).

Standard default PAM settings (Walz WinControl, Figure 2.6) were used including AF = 0.84. The ETR was then corrected for actual $A_{465\ nm}$ by $rETR \times A_{465}/A_F$ = ETR.

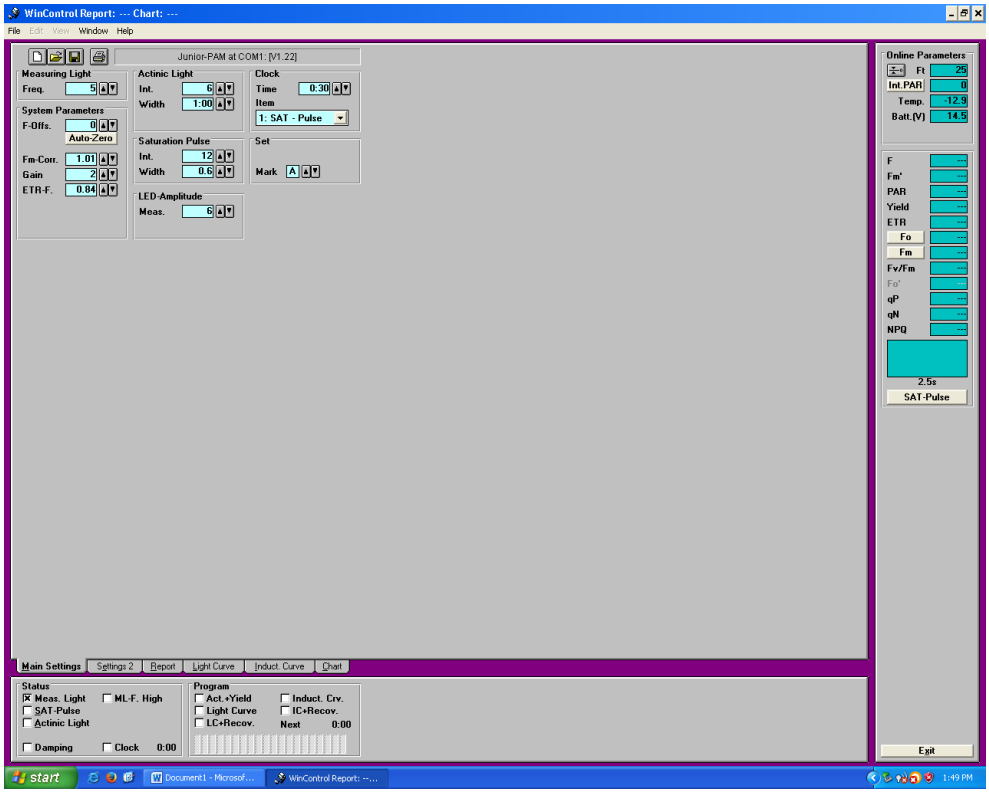

Figure 2.7 Walz WinControl Software showing standard settings (Settings 1)

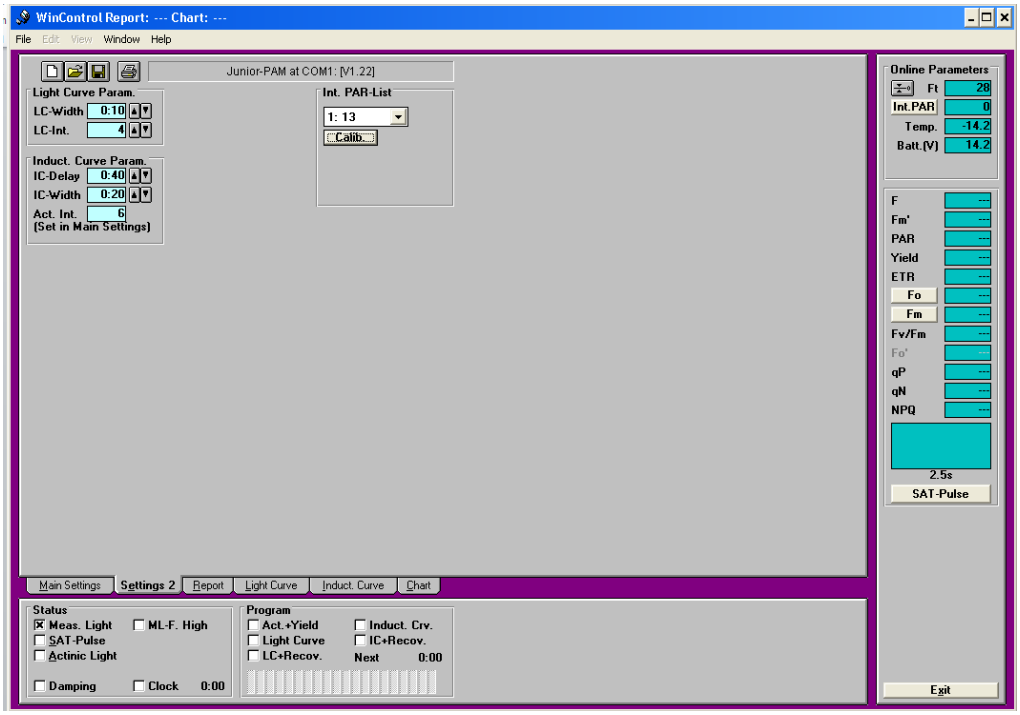

Figure 2.8 Walz WinControl Software showing standard settings (Settings 2)

Photosynthesis is generally very sensitive to physiological stress. PAM fluorescence technology is the simplest way of monitoring photosynthesis and is able to measure effects after very short exposure times and so it particularly suited to monitoring the immediate effects of a toxin. The basic experiment using a PAM machine is a rapid light curve to determine the response of a plant to a stepwise range of irradiances (White and Critchley, 1999; Ritchie and Bunthawin, 2010a, 2010b; Ritchie 2010; Ritchie, 2012). Sets of PAM light curve measurements took about 88 s to complete with 10 s between saturating flashes of light (0.8 s duration). The measuring light values were in order of increasing intensity. The key parameter measured with a PAM is an Apparent Photochemical Yield which is measured by fluorescence Induction (Genty, *et al*., 1989; van Kooten and Snel, 1990; Schreiber, *et al*., 1995). Apparent Photochemical Yield or simply Yield is usually designated (Y) or, more rarely (ΦPSII) to emphasize that the fluorescence yield of photosystem II (PSII) is being measured.

Yield is a measure of the proportion of incident photons that are actually used for electron transport by the photosystems of the chloroplasts of a plant. Maximum is about 0.7 to 0.8. The Yield is very sensitive to stress on the plant. The Electron Transport Rate (ETR) is an estimate of P_g and is proportional to the product of the Yield and the Irradiance and is defined as,

$$ETR = Y \times E \times 0.5 \times Abt \qquad \text{Equation 2.1}$$

where, Y is the effective quantum yield,

E is the irradiance (μmol m^{-2} s^{-1} PPFD),

0.5 is the PSI/PSII allocation factor (0.5) allows for about 50% of quanta being absorbed by PSII (Melis, 1989).

Abt in Equation 2.1 is the Leaf Absorptance Factor (Abt) which is usually assumed to be 0.84 but in conjunction with Equation Pty, Ltd (NSW, Australia) in this study the recently developed the RAT (Reflectance/Absorptance/Transmission) meter which can measure absorptance experimentally was used (Ritchie and Runcie, 2014). Absorptances of vascular plants are often considerably different to the standard value (Abt_F) of 0.84 (McCree, 1972; Björkman and Demmig, 1987; Ritchie and Runcie, 2014). Measurements of absorptances of juvenile and adult oil palms in the present study (Apichatmeta and Ritchie, 2016; Apichatmeta, *et al.*, 2016) agreed with previous studies and showed that blue light absorptance of oil Palms was much higher than the default value of $Abt_F = 0.84$ (Ritchie and Runcie, 2014).

2.3.2 Absorptance Measurements – the RAT

The RGB (Red – Green - Blue) - diode based leaf absorptance meter (RAT) (Reflectance –Absorptance - Transmission) was designed by Dr John Runcie (Equation Pty Ltd, Umina Beach, Australia) for the measurement of absorptance of leaves at the same blue light wavelengths as used by PAM fluorometers equipped with a blue-diode light source.

In this study actual absorptance measurements were used instead of the standard default absorptance value (Abt_F) of 0.84 used in many PAM studies to calculate the actual ETR of oil palms. The Blue-RAT was designed to be a simple, portable device for making relevant absorptance readings of leaves that would be more generally useable than the cumbersome and often unavailable Taylor Sphere method for measuring absorptances (Ritchie and Runcie, 2014). Absorptance is calculated as: Abt% = 100-T%-R%. The machine

is calibrated using a black card and a white standard surface. The RAT had been successfully applied to oil Palm seedlings and adult plants (Ritchie and Runcie, 2014) but in the present study more extensive measurements were made (Apichatmeta and Ritchie, 2016; Apichatmeta, *et al.*, 2016).

Modelling Electron Transport Rate vs Irradiance

Plots of Yield (Y) vs Irradiance (E) show that the data fitted a simple exponential decay curve of the form $y = e^{-x}$ (Ritchie, 2010). It follows from the finding that plots of Y vs E obey a simple exponential decay function that plots of ETR vs E should obey an exponential function known as the waiting-in-line model (probability density function or exponential waiting time distribution of the form $y = xe^{-x}$) (Ritchie and Bunthawin, 2010a, 2010b; Ritchie, 2012; Ritchie, 2014).

This is because the electron transport rate is directly proportional to the product of Yield and the number of photons absorbed by the photosynthetic apparatus. A form suitable for modelling photosynthesis that is easy to fit using non-linear least squares methods is (Ritchie, 2008; Ritchie and Bunthawin, 2010a, 2010b; Ritchie, 2012; Ritchie, 2014):

$$ETR = \frac{ETR_{\max}.\mathrm{E}}{\mathrm{E}_{\mathrm{opt}}} \mathrm{e}^{1-\mathrm{E}/\mathrm{E}_{\mathrm{opt}}} \qquad \text{Equation 2.2}$$

where, ETR is the electron transport rate,

E is the irradiance (μmol photons m^{-2} s^{-1}),

E_{opt} is the optimum irradiance, and

ETR_{max} is the maximum gross photosynthesis.

The maximum photosynthetic efficiency (α_0) is the initial slope of the curve at E = 0 ($\alpha_0 = e \times ETR_{max}/E_{opt}$). The half-maximum photosynthesis ($ETR_{half\text{-}max}$) is reached at $0.23196 \times E_{opt}$ and photosynthesis is also inhibited by 50% at $2.67341 \times E_{opt}$.

Expressions of Non-Photochemical Quenching (qN and NPQ)

Two slightly different equations are used to express non-photochemical quenching in plants (Genty, *et al*., 1989; Ritchie and Bunthawin, 2010a, 2010b; Ritchie, 2012) and are calculated automatically by the PAM Walz WinControl. qN and NPQ are expressions of the amount of waste heat loss in PSII and are measures of the pmf across the thylakoid membrane and the activity of the xanthophylls cycle in the thylakoid membrane. In general in vascular plants, NPQ >qN.

2.4 Calculation

Theory

The fluorescence yield was calculated by the WinControl program the effective quantum yield (Y), ranges from 0 to 1 (maximum usually no higher > 0.85). Experimentally that if Y is plotted irradiance (*E*),
it follows a simple exponential decay function from

$$y = e^{-kx} \qquad \text{Equation 2.3}$$

where Y is the effective quantum yield,
Y_{max} is the effective quantum yield at theoretical zero irradiance,
Kx is a scaling constant and
E is the irradiance.

The ETR is an estimate of P_g and is defined as ETR (mol m^{-2} s^{-1} PPFD),

ETR = Y x *E* x (PSI/PSII allocation factor) x (leaf absorptance factor) Equation 2.4

Where Y : effective quantum yield,
E : irradiance (mol m^{-2} s^{-1} PPFD) (Light expressed as number of photons),
PSI/PSII allocation factor (0.5) and
leaf absorptance factor (0.84)

ANOVA and the Tukey test procedure on the photosynthetic parameters were used to identify significant differences between Control, NH_4Cl, $NaNO_3$, KCl and $CaHPO_4$ treatments. Cochran and Snedecor (1989) was used as the standard statistical reference.

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2.5 Analysis

Photosynthesis and Nutrients

Some simple nutrient studies were part of the project but because of time restrictions such studies were restricted to seedlings. Seedlings were readily available and were grown in pots of potting mix and fed standard NPK-type fertilizers. A laboratory version of a standard NPK fertilizer was used where manipulations of nutrient content are required. Simple nutrient experiments on photosynthetic responses were done using a PAM machine.

In general, photosynthetic responses to nitrogen sources (ammonia, urea and nitrate) were expected to be easily detectable and very rapid (Nelson and Sommers, 1973). Phosphate effects are generally difficult to detect using a PAM machine.

PAM is known to be a sensitive detector of iron (Fe) deficiency because Fe deficiency has direct effects on photosynthesis. Rapid light curves were performed on plants 3 hours after watering with an experimental nutrient solution.

CHAPTER 3

Results

3.1 Reflectance, Transmission and Absorptance of Oil Palm

Spectral characteristics of selected plants at 465 nm (blue diode), $R_{465\ nm}$, $T_{465\ nm}$, and Abt_{465nm} – Reflectance, Transmittance, and Absorptance at 465 nm, respectively (Ritchie and Runcie, 2014). The results found in the present study are shown in Table 3.1 Actual absorptance measurements of plants are superior to simply assuming a default absorptance (Abt_F) of 0.84 (Ritchie and Runcie, 2014). Absorptance information on seedlings was not previously available, but the measurements in juveniles and adults made in the present study are similar to those previously published by Ritchie and Runcie (2014). Absorptances of seedlings had not been measured previously.

Table 3.1 Leaf Absorptance Characteristics of Oil Palm (*Elaeis guineensis*)

Species	**$R_{465\ nm}$ [%]**	**$T_{465\ nm}$ [%]**	**$Abt_{465\ nm}$ [%]**	**Chlorophyll *a* Content Chl *a* (mg/m^2)**
Oil Palm seedling	2.24 ± 0.30	0.10 ± 0.210	96.66 ± 0.49	154.4 ± 15.22
Oil Palm juvenile	2.02 ± 0.09	0.16 ± 0.060	97.83 ± 0.24	242.8 ± 23.38
Oil Palm adult	0.12 ± 0.01	0.10 ± 0.001	99.98 ± 0.13	474.0 ± 43.52

(n = 16, ±95% CL)

Seedling/Juvenile leaves and adult leaves are very different in morphology (see pictures in Chapter 2; Table 3.1). The adult leaves have a reflectance of almost zero compared to juvenile and seedling leaves. Absorptance of adult leaves is almost 100% and compared to leaves of most other plants (Ritchie and Runcie, 2014) even the absorptances of juvenile and seedling leaves are very high. Essentially oil Palm leaves are optically black under blue light; they absorb practically all incident light. The chlorophyll *a* content of adult leaves is about twice that found in juvenile leaves on a surface area basis. The amount of chlorophyll *a* per unit surface area is also lower than juvenile plants.

3.2 PAM Parameters

PAM parameters are shown in Tables 3.2, 3.3 and 3.4. ETR was measured using the PAM machine on seedling, juvenile and adult oil Palm. 16 replicate leaves of each growth stage were used: the adult oil Palms growing on the Phuket campus were used for the measurements on adult plants. Absorptance measurements from Table 3.1 were used to correct rETR to ETR (Ritchie and Runcie, 2014). Actual absorptances of oil Palm leaves (Table 3.1) are very different to the default value (Abt_F) used by the Walz software (0.84) and so underestimates the actual ETR by about 13.4% in the case of seedlings and juveniles and about 15.9% in the case of adult plants (Table 3.1).

PAM measurements of photosynthetic parameters in *Elaeis guineensis* show pronounced diel behavior in photosynthesis with effective yield, ETR, and photosynthetic efficiencies changing during the course of the day. Measurements of effective yield, relative electron transport rate (rETR), photosynthetic efficiency (α_0), and non-photochemical (qN and NPQ) quenching were made on solar time and routinely based on 16 leaves for each growth stage.

Efficiency of Yield values of the 3 life stages in oil palm were highest in seedling 0.71 ± 0.035 followed by juvenile as 0.698 ± 0.020 (juvenile and seedling were not significantly different) and 0.629 ± 0.023 in the adult plant. The maximum Yield efficiency occurs around midday (Tables 3.2-3.4, Figure 3.1). The Electron Transport Rate (μmol m^{-2} s^{-1}) was highest in adult as 65.29 ± 1.067 μmol e^- m^{-2} s^{-1}, in juveniles equal to 44.57 ± 0.786 μmol e^- m^{-2} s^{-1} and in seedlings, 33.39 ± 0.663 μmol e^- m^{-2} s^{-1}. The largest difference among these three stages was in seedlings which have the highest value at 09:00, compared to adult and juvenile at 12:00 (Tables 3.2, 3.3 and 3.4, Figure 3.2).

Adult oil palm is able to use higher optimum irradiance than juveniles and seedlings. For adult the E_{opt} was highest at 15:00 as 816.8 ± 45.64 μmol e^- m^{-2} s^{-1}, whereas juveniles had a photosynthetic rate of only 580.6 ± 23.82 μmol e^- m^{-2} s^{-1} at the same time. The seedling had their highest optimum irradiance value at 09:00 as 511.9 ± 20.1 μmol e^- m^{-2} s^{-1} (Tables 3.2, 3.3 and 3.4, Figure 3.3).

Table 3.2 Parameters using PAM of Adult Oil Palm (Means ± 95% confidence limits)

Parameter	Solar Time				
	06:00	**09:00**	**12:00**	**15:00**	**18:00**
Yield (Y)	0.576 ± 0.030	0.603 ± 0.026	0.629 ± 0.023	0.556 ± 0.026	0.531 ± 0.017
Optimum E (μmol photon m^{-2} s^{-1})	619.4 ± 34.48	764.0 ± 17.37	631.6 ± 24.98	816.8 ± 45.64	706.6 ± 31.14
Electron transport rate (μmol e^{-} m^{-2} s^{-1})	35.54 ± 1.14	63.15 ± 0.73	65.29 ± 1.07	61.24 ± 1.68	36.88 ± 0.87
Gross photosynthesis (μmol O_2 mg Chla^{-1} h^{-1})	67.48 ± 2.17	119.9 ± 1.39	126.1 ± 3.14	117.1 ± 3.20	83.81 ± 3.75
Photosynthetic Efficiency (α_0)					
- Surface Area Basis	0.196 ± 0.024	0.225 ± 0.014	0.291 ± 0.016	0.204 ± 0.014	0.182 ± 0.02
- Chl *a* basis (m^2 g Chl a^{-1})	0.773 ± 0.039	0.894 ± 0.034	0.918 ± 0.024	0.835 ± 0.028	0.693 ± 0.038
Non Photochemical Quenching					
- qN_{max}	0.567 ± 0.025	0.744 ± 0.031	0.774 ± 0.042	0.638 ± 0.022	0.563 ± 0.023
- NPQ_{max}	1.184 ± 0.083	1.482 ± 0.102	1.287 ± 0.094	1.141 ± 0.07	1.139 ± 0.103

Table 3.3 Parameters using PAM of Juvenile Oil Palm (Means ± 95% confidence limits)

Parameter	Solar Time				
	06:00	**09:00**	**12:00**	**15:00**	**18:00**
Yield (Y)	0.606 ± 0.025	0.681 ± 0.019	0.698 ± 0.020	0.634 ± 0.031	0.661 ± 0.031
Optimum E (μmol photon m^{-2} s^{-1})	619.4 ± 34.48	764.0 ± 17.37	631.6 ± 24.98	816.8 ± 45.64	706.6 ± 31.14
Electron transport rate (μmol e^- m^{-2} s^{-1})	19.47 ± 0.616	31.89 ± 0.691	44.57 ± 0.786	41.63 ± 1.022	30.95 ± 1.332
Gross photosynthesis (μmol O_2 mg Chl a^{-1} h^{-1})	72.17 ± 2.283	118.2 ± 2.562	165.2 ± 2.913	154.4 ± 3.787	114.8 ± 4.937
Photosynthetic Efficiency (α_0)					
- Surface Area basis	0.183 ± 0.01	0.228 ± 0.01	0.244 ± 0.009	0.195 ± 0.009	0.176 ± 0.016
- Chl *a* basis (m^2g Chl a^{-1})	0.680 ± 0.018	0.744 ± 0.015	0.793 ± 0.012	0.723 ± 0.015	0.652 ± 0.016
Non Photochemical Quenching					
- qN	0.778 ± 0.030	0.824 ± 0.032	0.897 ± 0.017	0.732 ± 0.020	0.838 ± 0.017
- NPQ	1.624 ± 0.119	1.754 ± 0.125	1.920 ± 0.060	1.287 ± 0.061	1.859 ± 0.067

Table 3.4 Parameters using PAM of Seedling Oil Palm (Means ± 95% confidence limits)

Parameter	Solar Time				
	06:00	**09:00**	**12:00**	**15:00**	**18:00**
Yield (Y)	0.65 ± 0.03	0.67 ± 0.04	0.71 ± 0.04	0.67 ± 0.03	0.66 ± 0.03
Optimum E (μmol photon m^{-2} s^{-1})	619.4 ± 34.48	764.0 ± 17.37	631.6 ± 24.98	816.8 ± 45.64	706.6 ± 31.14
Electron transport rate (μmol e^- m^{-2} s^{-1})	25.34 ± 1.21	33.39 ± 0.66	26.79 ± 1.52	17.66 ± 0.77	19.90 ± 0.55
Gross photosynthesis (μmol O_2 mg Chl a^{-1} h^{-1})	147.7 ± 7.03	194.56 ± 3.86	156.13 ± 8.84	102.90 ± 4.48	116.0 ± 3.19
Photosynthetic Efficiency (α_0)					
- Surface Area basis	0.15 ± 0.01	0.19 ± 0.005	0.207 ± 0.015	0.178 ± 0.013	0.105 ± 0.007
- Chl *a* basis (m^2 g Chl a^{-1})	0.296 ± 0.019	0.427 ± 0.011	0.508 ± 0.019	0.377 ± 0.017	0.309 ± 0.017
Non Photochemical Quenching					
- qN	0.598 ± 0.036	0.470 ± 0.038	0.728 ± 0.023	0.485 ± 0.038	0.523 ± 0.043
- NPQ	1.072 ± 0.126	0.533 ± 0.050	1.256 ± 0.052	0.601 ± 0.076	0.758 ± 0.101

The results on efficiency of yield value of the 3 life stages in oil palm show that Yield was highest in seedling 0.71 ± 0.035 followed by juvenile as 0.698 ± 0.020 (juvenile and seedling were not significantly different) and as 0.629 ± 0.023 in the adult plant. The maximum efficiency occurs around midday (Tables 3.2, 3.3 and 3.4, Figure 3.1). The Electron Transport Rate (μmol m^{-2} e^{-} s^{-1}) was highest in adult as 65.29 ± 1.067 μmol e^{-} m^{-2} s^{-1}, in juveniles equal to 44.57 ± 0.786 μmol e^{-} m^{-2} s^{-1} and in seedlings, 33.39 ± 0.663 μmol e^{-} m^{-2} s^{-1}. The largest difference among these three stages was in seedlings which have the highest value at 09:00, compared to adult and juvenile at 12:00 (Tables 3.2-3.4, Figure 3.2).

Adult oil palm is able to use higher optimum irradiance than juveniles and seedlings. For adult the E_{opt} was highest at 15:00 as 816.8 ± 45.64 μmol e^{-} m^{-2} s^{-1}, whereas juveniles had a photosynthetic rate of only 580.6 ± 23.82 μmol e^{-} m^{-2} s^{-1} at the same time. The seedling had their highest optimum irradiance value at 09:00 as 511.9 ± 20.1 μmol e^{-} m^{-2} s^{-1} (Tables 3.2-3.4, Figure 3.3).

Gross photosynthesis was found to be highest in seedlings at about 09:00 whereas juvenile and adult plants had the highest gross photosynthesis at noon as 194.6 ± 3.86 μmol e^{-} m^{-2} s^{-1}, 165.2 ± 2.91 μmol e^{-} m^{-2} s^{-1} and 126.1 ± 3.14 μmol O_2 mg Chl a^{-1} h^{-1}, respectively (Tables 3.2-3.4, Figure 3.4).

Photosynthetic Efficiency (α_0) can be expressed in two ways: on a Surface Area basis and a Chlorophyll *a* basis (m^2 g Chl a^{-1}). The Photosynthetic Efficiency shows the same pattern for both parameters among the 3 stages of oil palm: the apparent photosynthetic efficiency was highest at noon. The highest value was found in adult equal to 0.255 ± 0.006 (SA basis) and 1.186 ± 0.018 m^2 g Chl a^{-1}, next was juvenile oil palm as 0.228 ± 0.010 and 0.993 ± 0.032 m^2 g Chl a^{-1} and the last was seedling as 0.197 ± 0.008 and 0.918 ± 0.094 m^2 g Chl a^{-1} (Tables 3.2-3.4, Figure 3.5).

Non Photochemical Quenching is expressed as qN and NPQ parameters. Among these three stages of oil palms, the highest measurement for both qN and NPQ occurred in juvenile plants at midday as 0.897 ± 0.017 and 1.920 ± 0.060 respectively. Seedlings ranked second with qN equal to 0.728 ± 0.023 and NPQ equal to 1.256 ± 0.052. The lowest values were found in the adult where qN was 0.774 ± 0.042 and NPQ was 1.482 ± 0.102. However, qN in adult had a maximum value at 09:00 but NPQ was highest at noon (Tables 3.2-3.4, Figure 3.6). In contrast to photosynthesis and photosynthetic efficiency, qN and NPQ did not change over the course of the day in a clearly systematic way. There is no obvious pattern in qN and NPQ over the course of the day.

The pattern of photosynthesis parameters during all day measurement is shown in following Figures: Photosynthetic Properties of Seedling, Juvenile and Adult oil Palm Plants.

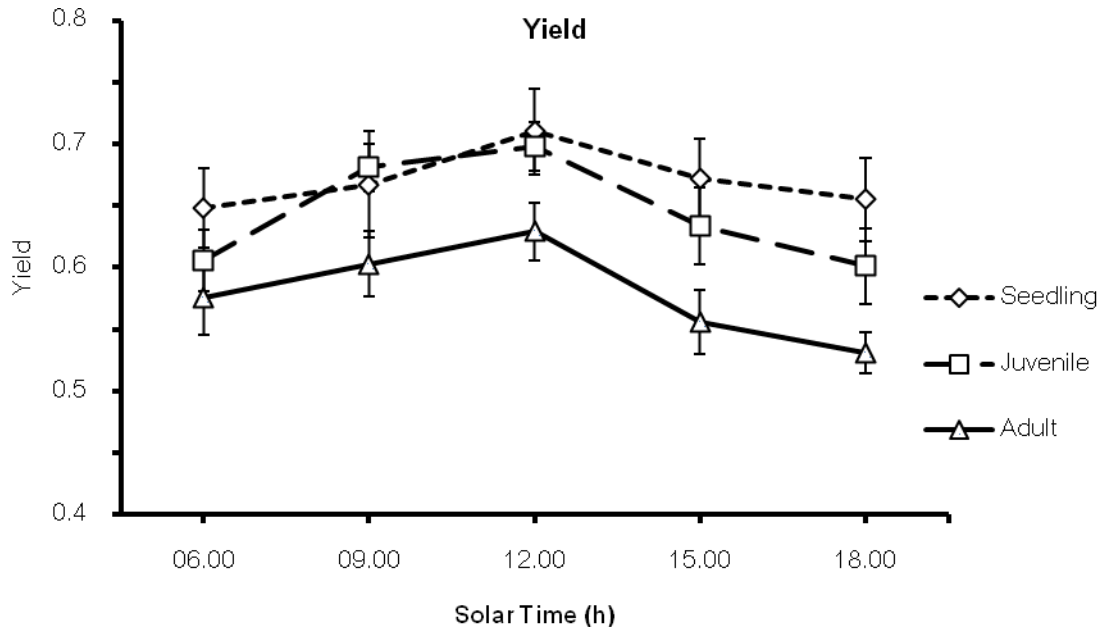

Figure 3.1 Maximum effective photosynthetic yield (Y) vs Solar Time of Oil Palm leaves collected over the day. Data reported as means ± 95% confidence limits. Yield tends to reach maximum in the middle of the day.

The maximum effective photosynthetic yield was low in the early morning and increased during the day reaching a peak around noon for all three stages of oil palm, after that Yield declined until the end of the experiment time at 18:00. Meanwhile, the photosynthetic yield maximum in juvenile and seedling had rather similar values in the morning, but slight declines in the afternoon occurred in the seedling. Among three stages, seedling oil palm had the highest maximum effective photosynthetic yield.

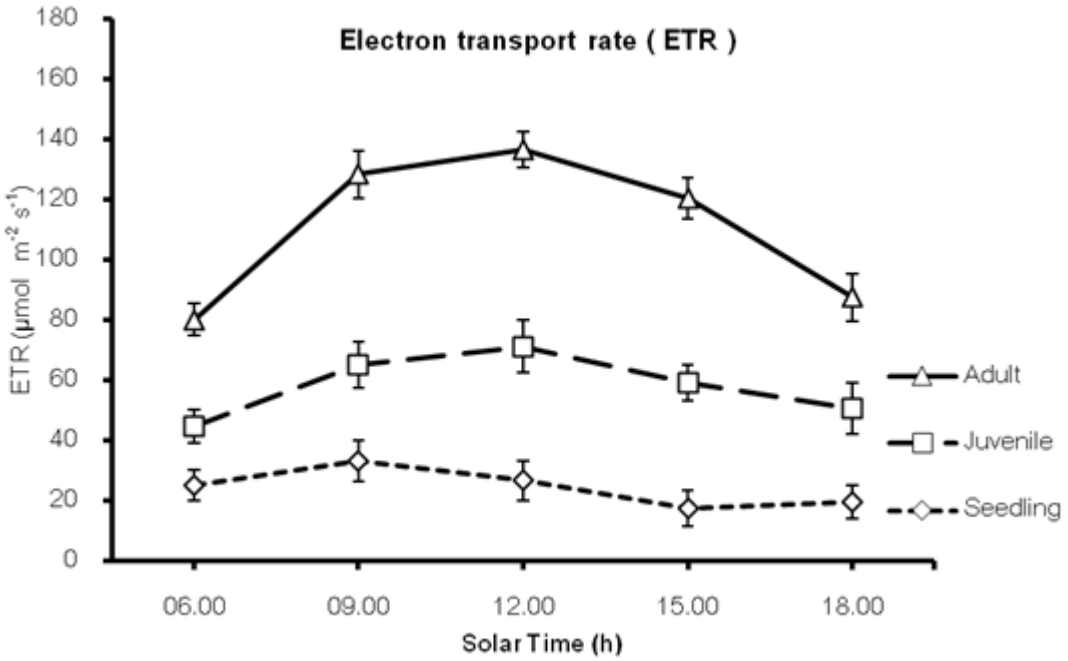

Figure 3.2 Electron transport rate (ETR) vs Solar Times of Oil Palm of each type of leaves of collected over the day. Pulse amplitude modulation (PAM) light curve data was based on 16 leaves and 5 different times. ETR_{max} is presented as means ± 95% confidence limits. ETR of juvenile and adult plants maximizes in the middle of the day but in seedlings is optimum at 09:00 and then declines.

As shown in Figure 3.2, the electron transport rate pattern was rather similar between juvenile and adult, with low rates in the early morning and increasing to a maximum point at midday then decreasing until end of the experiment at dusk. Gradual changes were occurring in juvenile compared to the adult where the diurnal effect was larger. A dissimilar pattern was found in seedling: the ETR had the peak value at 09:00 and declined to the lowest value at 15:00.

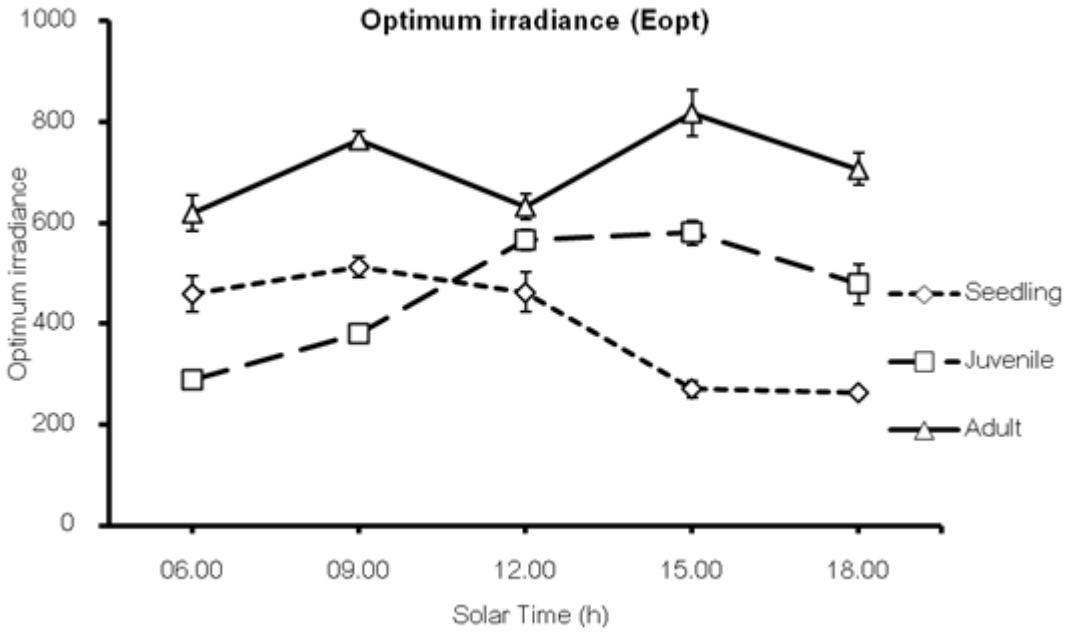

Figure 3.3 Optimum irradiance (E_{opt}) vs Solar Time for Oil Palm leaves collected over the course of daylight. Pulse amplitude modulation (PAM) light curve data are based on 16 leaves and 5 different times. E_{opt} presented as means ± 95% confidence limits.

Optimum irradiance in seedling was found at 09:00 and followed by juvenile at about noon, and the optimum occurred in the adult at 15:00. Optimum irradiance in adult seems to reach two high peaks as at 09:00 and at 15:00 as the second peak. There seems to be some midday depression of E_{opt} in adults in the middle of the day. This pattern was not found in seedlings and juveniles. Optimum irradiance in seedlings decreased after early morning (09:00) whereas this did not occur in juveniles or adults.

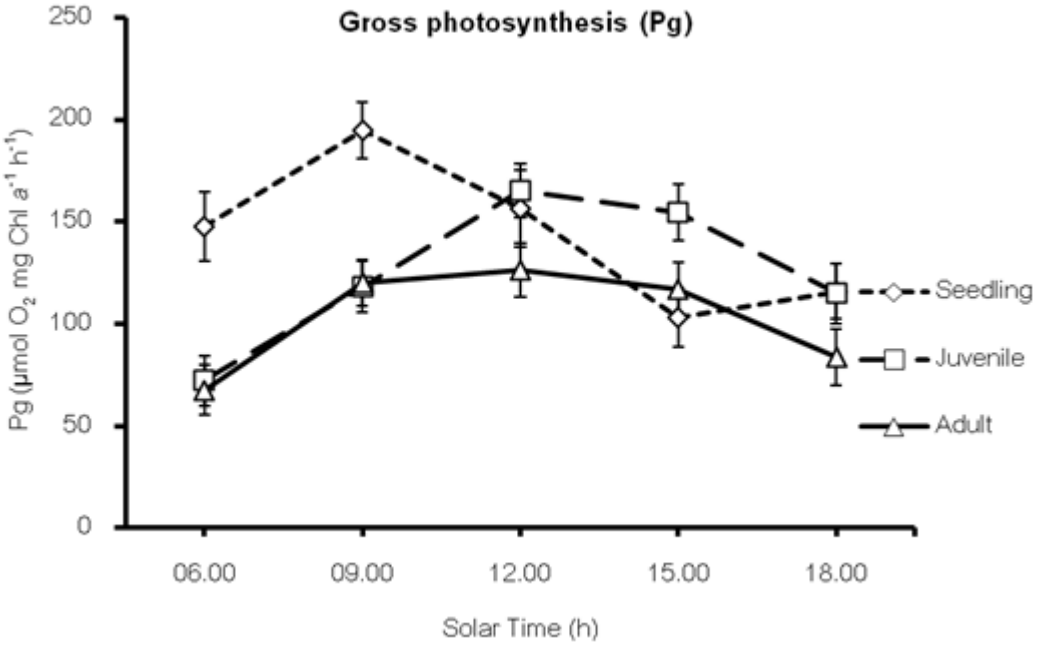

Figure 3.4 Gross photosynthesis (Pg) vs Time of Solar day of *Elaeis guineensis* leaves. Pg_{max} displayed as means ± 95% confidence limits.

The Maximum Gross photosynthesis (Pg_{max}) increased from the morning and reached the peak level in different times among the three growth stages of oil palm and in all three classes of plants there was a decline in the afternoon. Seedlings had the highest Pg_{max} at 09:00 whereas juvenile and adult had highest value at noon; however, there was an increase again after 15:00 in seedlings.

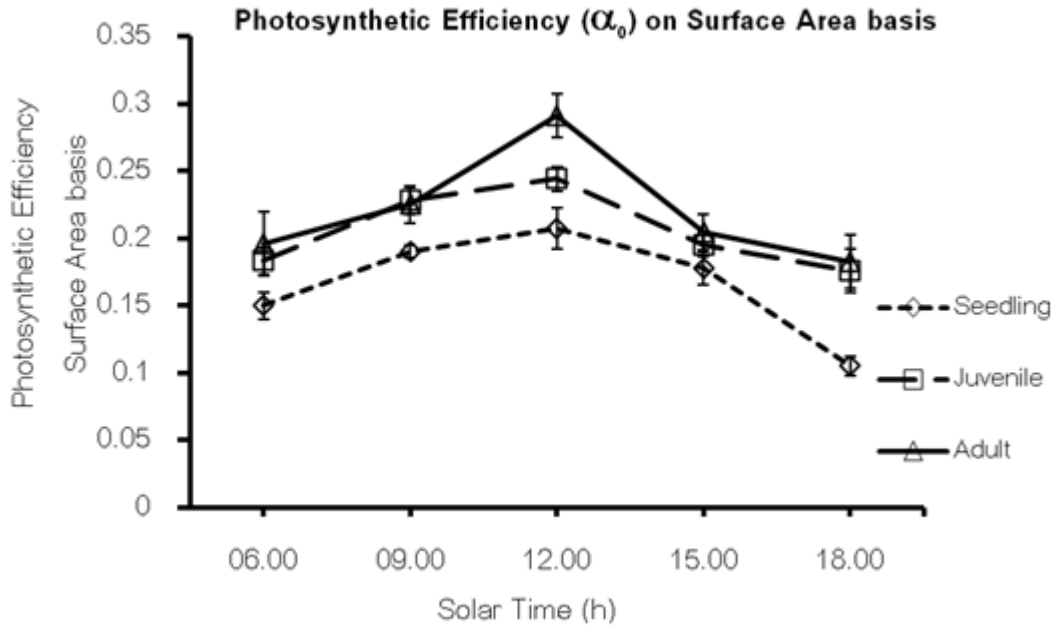

Figure 3.5 Photosynthetic Efficiency (α_0) on Surface Area basis vs Time of Solar day of *Elaeis guineensis* leaves. α_0 displayed as means ± 95% confidence limits.

As mentioned in the first part of the Result there are two ways of expressing Photosynthetic Efficiency (α_0). On a Surface Area basis photosynthetic efficiency of all three stages is very similar at each time of the day and the highest efficiency was found at 12:00. There was a gradual decline of photosynthetic efficiency after 12:00 until the end of measurements at 18:00.

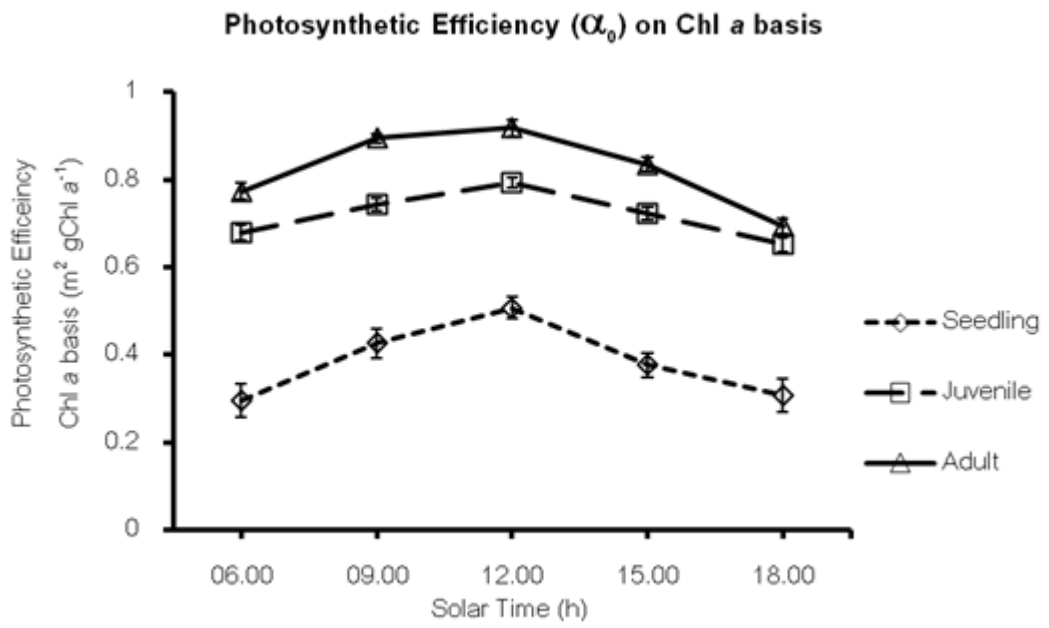

Figure 3.6 Photosynthetic Efficiency (α_0) on Chl *a* basis vs Times of Oil Palm leaves over the course of a solar day. The effect of the differences in chlorophyll content of the three classes of oil palm plants results in Figure 3.6 looking different to Figure 3.5. Pulse amplitude modulation (PAM) light curve data are based on 16 leaves and 5 different times. Photosynthetic Efficiency by Chlorophyll *a* basis expressed as means ± 95% confidence limits. Chlorophyll *a* basis of seedling, juvenile and adult plants all maximize in the middle of the day.

The photosynthetic efficiency indicated by chlorophyll *a* basis was parallel with the same data expressed on a surface area basis. The adult oil palm had the highest photosynthetic efficiency value among them while juveniles had slightly higher than seedlings. Photosynthetic efficiency of seedlings, juveniles and adults show the same characteristic diurnal pattern: efficiencies were low at daybreak and increased in the morning, the highest peak was found at midday and efficiency decreased in the afternoon.

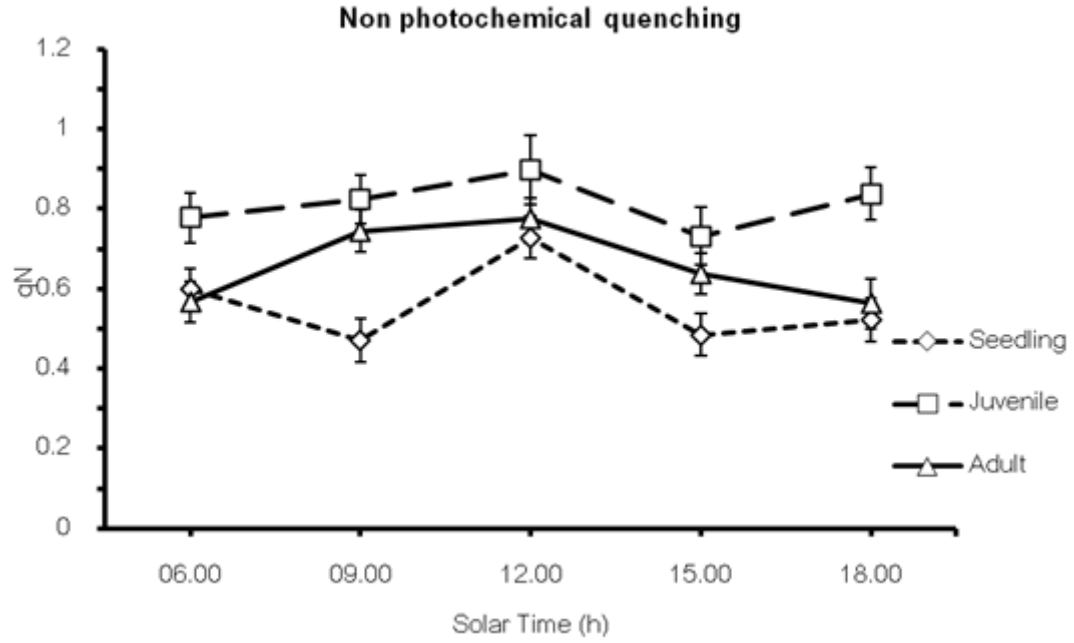

Figure 3.7 Non-photochemical quenching (qN**)** vs Times of *Elaeis guineensis* leaves.

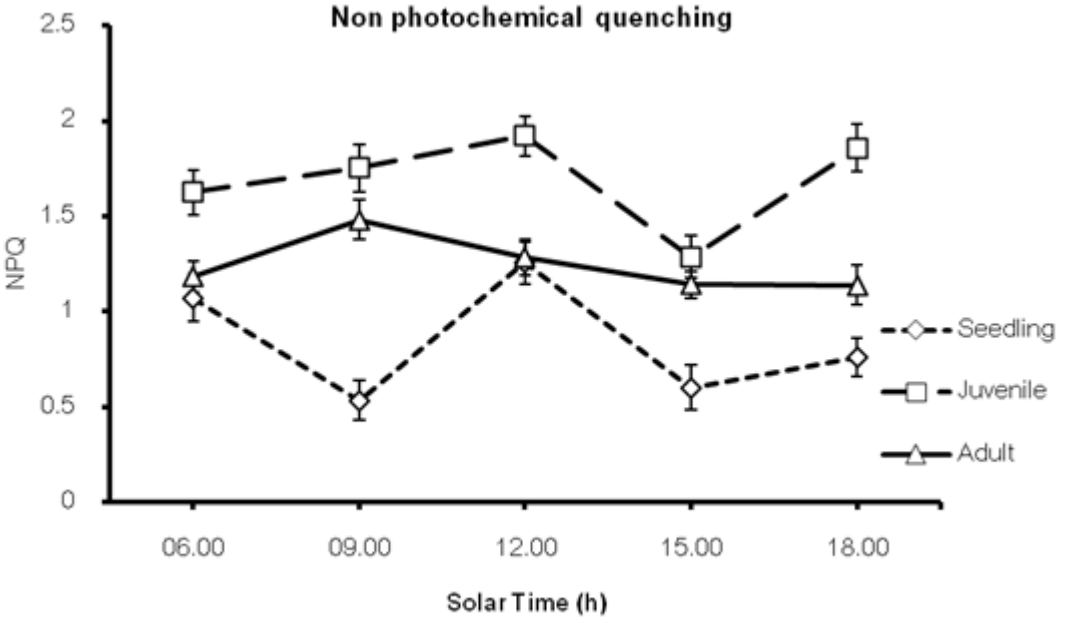

Figure 3.8 Non-photochemical quenching (NPQ) vs Time of Solar Day of *Elaeis guineensis* leaves.

Non-photochemical quenching can be expressed as qN and NPQ. qN_{max} and NPQ_{max} was calculated from non-linear least squares fits to non-photochemical quenching vs irradiance curves using a simple exponential saturation model (Ritchie 2008; Ritchie and Bunthawin, 2010a, 2010b; Ritchie 2012; Ritchie 2014). qN_{max} and NPQ_{max} at each time are expressed as means ± 95% confidence limits.

Rather high fluctuation of Non photochemical quenching was found in this study. No readily apparent pattern is found in qN or NPQ over the course of a day except for the observation the both qN and NPQ show little diurnal variation in the adult plants but qN and NPQ seem to fluctuate greatly in seedlings and juveniles compared to the adults where qN and NPQ appear to be more constant.

Juvenile oil palm expressed the highest qN and NPQ at midday and sharply declined at 15:00 and increased slightly again at the end of measurement at 6pm Solar time. A similar pattern was found in the seedling stage. Contrary to what was found in the adult, qN was moderately different between at 09:00 and at 12:00, but the highest values occurred at 12:00. NPQ of adult was highest at 09:00 and slowly decreased from 12:00 to sunset.

Experiments were run to attempt to detect short-term effects of added nutrients on PAM parameters of Oil Palm plants. When one or several nutrients are not in sufficient supply, this would be expected to have effects on the photosynthetic rates of plants and in general would reduce growth to below the potential set by similar environmental conditions with adequate mineral nutrition. PAM parameters include Yield, Optimum irradiance (E), Electron transport rate (ETR), Non photochemical quenching **(**qN and NPQ), these were compared in control Oil Palm seedlings and in seedlings offered additional N-sources, Potassium (K) and phosphate. The response of plants to changes in their K, P, and N supplies are known to be different in some respects (Clarkson and Hanson, 1980). Each result is shown graphically using Leaf Absorptance Characteristics in Table 3.1 and the Chl *a* per unit leaf area data to convert ETR to photosynthesis as µmol mg Chl a^{-1} h^{-1}.

Well-watered Oil Palm seedlings growing in pots were used as the controls. Plants were offered 10 mol m^{-3} NH_4Cl, $NaNO_3$, KCl and $CaHPO_4$ in the light. Plants were exposed to the added nutrients for 2–3 h before there photosynthetic characteristics were measured using the PAM machine. These experiments therefore measured short-term effects of the added nutrients and not the effects after several days which may be quite different.

Table 3.5 Parameter nutrients up take using PAM of Seedling Oil Palm (Means ± 95% confidence limits.

Parameter	Control	NH_4Cl	$NaNO_3$	KCl	$CaHPO_4$
Yield (Y)	0.704 ± 0.01	0.66 ± 0.01	0.688 ± 0.01	0.672 ± 0.01	0.658 ± 0.01
Electron transport rate (µmol m^{-2} s^{-1})	25.41 ± 1.16	14.00 ± 0.99	17.07 ± 0.59	15.04 ± 0.61	14.08 ± 0.74
Optimum E (E_{Opt})	376.7 ± 28.4	272.6 ± 28.90	324.0±17.07	290.6 ± 17.79	270.5 ± 19.26
Gross photosynthesis (µmol O_2 mg Chl a^{-1} h^{-1})	148.1 ± 6.77	87.40 ± 5.80	108.3 ± 3.43	87.05 ± 3.60	83.64 ± 4.28
Photosynthetic Efficiency (α_0)					
- Surface Area basis	0.183 ± 0.02	0.139 ± 0.02	0.154 ± 0.01	0.14 ± 0.01	0.124 ± 0.01
- Chl *a* basis (m^2gChl a^{-1})	1.069 ± 0.09	0.812 ± 0.10	0.983 ± 0.07	0.814 ± 0.06	0.758 ± 0.071
Non photochemical quenching					
- qN	0.45 ± 0.03	0.56 ± 0.04	0.47 ± 0.03	0.62 ± 0.03	0.70 ± 0.03
- NPQ	0.50 ± 0.05	0.67 ± 0.07	0.58 ± 0.04	0.70 ± 0.05	0.79 ± 0.07

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Added nutrients did have significant measureable effects on PAM parameters but the results need to be interpreted carefully. The added nutrients had little measureable effects on maximum yield but affected the *shape* of the photosynthesis vs. irradiance curves. The change in shape was the result of both E_{opt} and Pg_{max} changing in response to added nutrients. Optimum irradiance (E_{opt}) and maximum photosynthesis (ETR_{max} and Pg_{max}) were significantly lower compared to the controls. Photosynthetic efficiency, expressed on both surface area and chlorophyll bases also significantly decreased. Seedling oil Palm with over nutrient supply, particularly phosphate (PO_4^{3-}) express lower photosynthetic efficiency than the controls, however, all the added nutrients had an apparently suppressing effect on photosynthesis. This was not expected. Phosphate tends to be the limiting factor for oil palm plants photosynthesis rate, and followed by ammonium, nitrite and nitrate. Potassium has more effect to photosynthetic efficiency than sodium (Parkhill, *et al*., 2001).

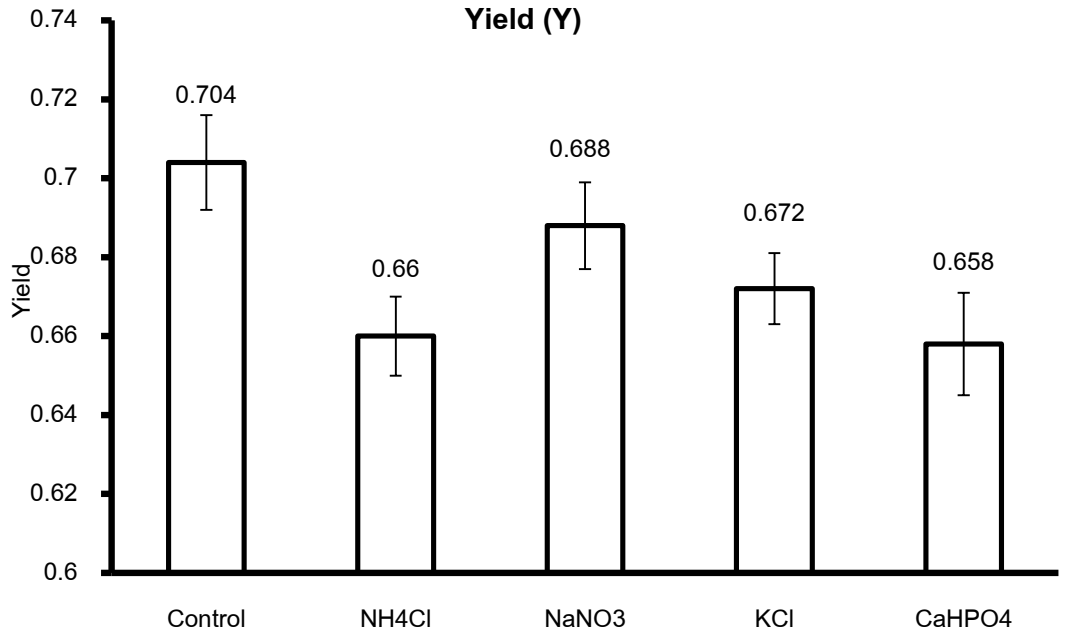

Figure 3.9 maximum effective photosynthetic yields (Y_{max}) of seedling oil palm with over nutrient supply. Control as normal environment condition. Maximum Yield is expressed as mean, error bar as ± 95% confidence limits.

Based on the result, seedling with added phosphate (HPO_4^{2-}) has significantly different effects on Y_{max} compared to the control, and also this phenomenon has found from seedling with added ammonium and potassium. The addition of nitrate had no significant effect compared to the control.

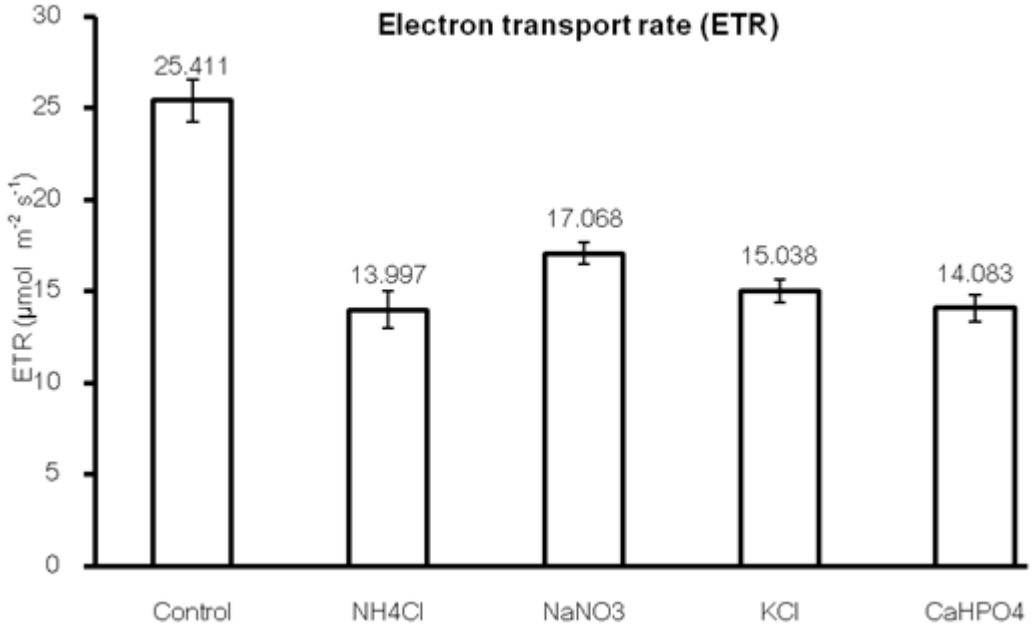

Figure 3.10 Maximum Electron transport rate (ETR_{max}) of seedling oil palm with over different supplied nutrients compared to the control plants. Control was the normal environmental condition for the seedlings. ETR_{max} expressed as mean ± 95% confidence limits.

Added NH_4Cl, $NaNO_3$, KCl and $CaHPO_4$ all had significant effects on ETR_{max} compared to the control but in every case an inhibitory effect was found. All were lower than the control. The pattern of negative effect from addition nutrients was similar to the pattern of Y_{max} pattern. $NaNO_3$ had the least effect, followed by potassium-added, phosphate-added and ammonium-added.

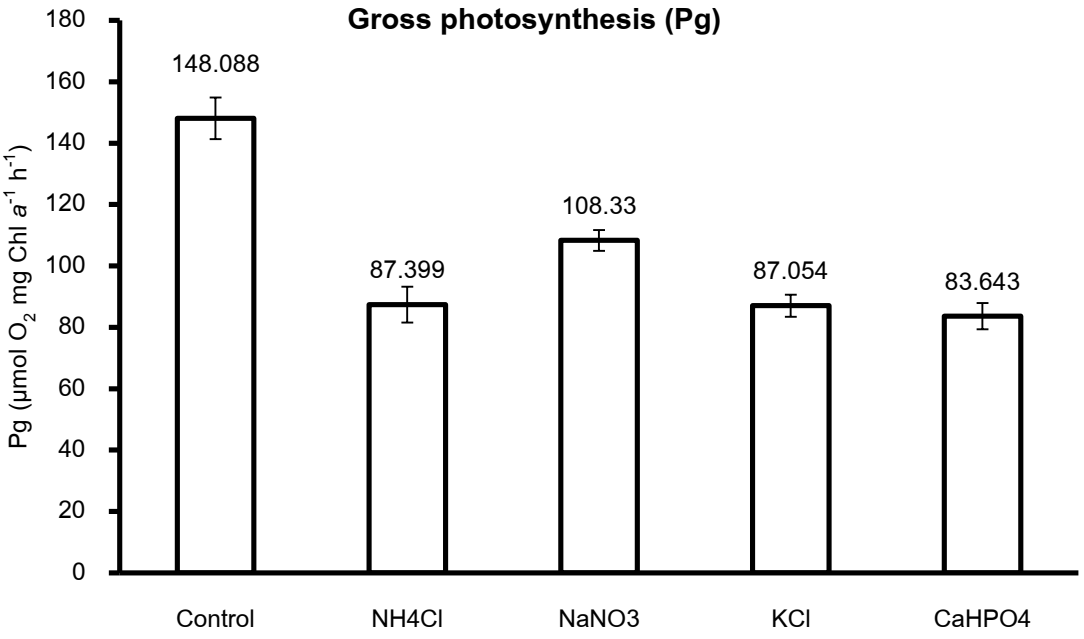

Figure 3.11 Maximum Gross photosynthetic (Pg_{max}) of seedling oil palm supplied with different nutrient compared to the control plants expressed on a chlorophyll basis. Pg_{max} is expressed as mean ± 95% confidence limits.

Correspondingly, with the results found with Y_{max} and ETR_{max}, Pg_{max} from nutrient-enrichment seedlings were obviously lower than the control. Added Nitrate had the lowest effect on ETR_{max} compared to the other three nutrients-fed seedlings, moreover, it had a significantly different effect compared to the phosphate-fed seedling.

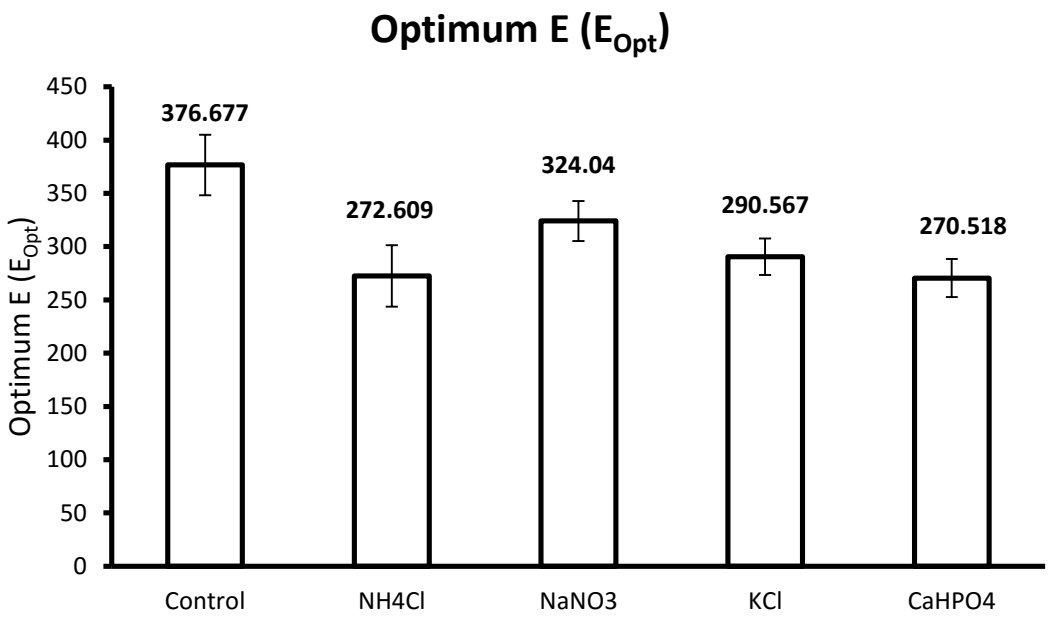

Figure 3.12 Optimum irradiance (E_{opt}) of seedling oil palm provided with different nutrients compared to the control. Control as normal environment condition. Optimum irradiance (E_{opt}) expressed as average value, error bar as ± 95% confidence limits.

The optimum irradiance (E_{Opt}) in both adult, juvenile and seedling oil palm plants are typical of sun plants. Oil palm seedling need less PPFD irradiance compared to adult oil palm ($E_{Opt} \approx 270$ vs 816 µmol quanta m^{-2} s^{-1}) on 15:00. The optimum irradiance decreased in plants fed ammonium, nitrate, potassium and phosphate indicate that the added nutrients changed the *shape* of the ETR vs irradiance curves.

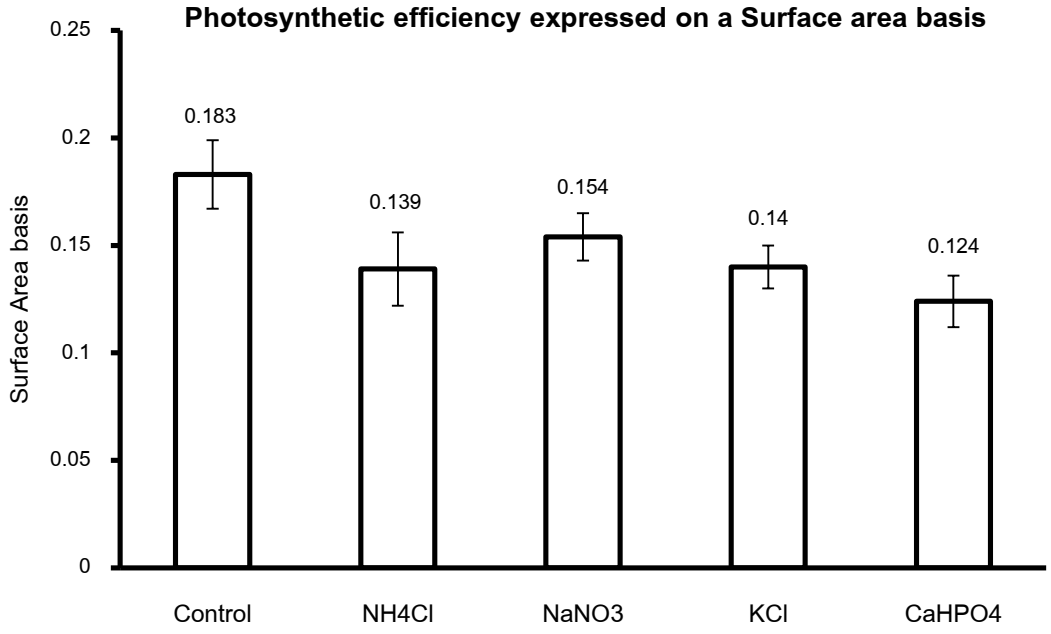

Figure 3.13 Photosynthetic Efficiency (α_0) expressed on a surface area basis of seedling oil palms supplied different nutrients and compared to the control. The control plants were kept under normal environmental conditions. α_0 is expressed as mean ± 95% confidence limits.

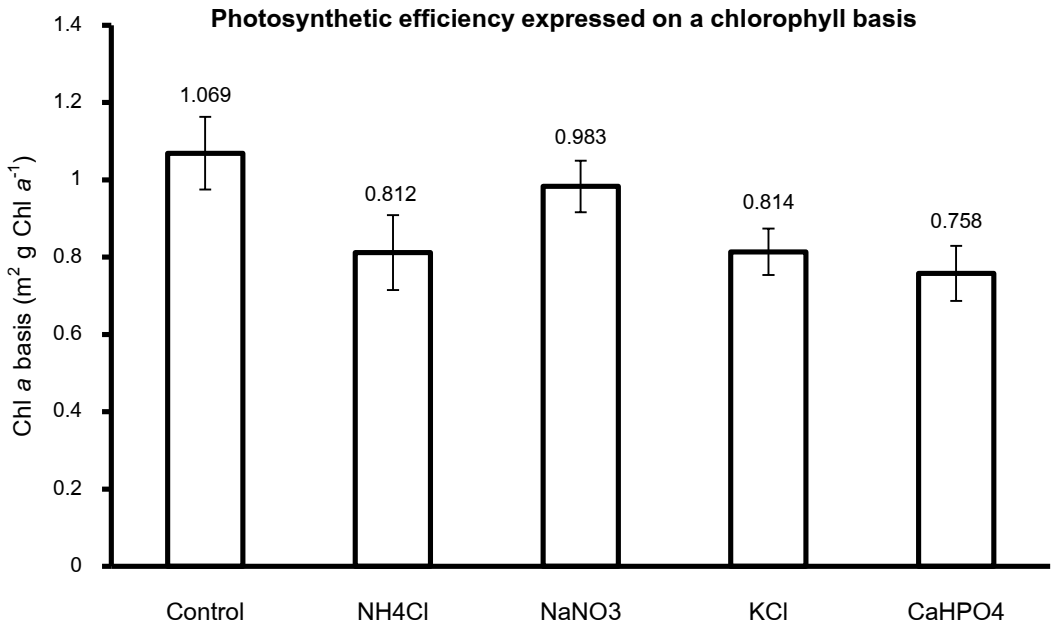

Figure 3.14 Photosynthetic Efficiency (α_0) expressed on a Chlorophyll *a* basis of seedling oil palms supplied different nutrients and compared to the control. The control was plants were kept under normal environmental condition. α_0 is expressed as mean ± 95% confidence limits.

Potential photosynthesis by seedling oil palm plants is very high on both a leaf surface area and Chlorophyll *a* basis. Oil palm adult leaves have exceptionally high efficiency in photosynthesis ($\alpha_0 \approx 0.22$ which is higher than in most leaves). All the added nutrients used in the study lead to a decrease in apparent photosynthetic efficiency.

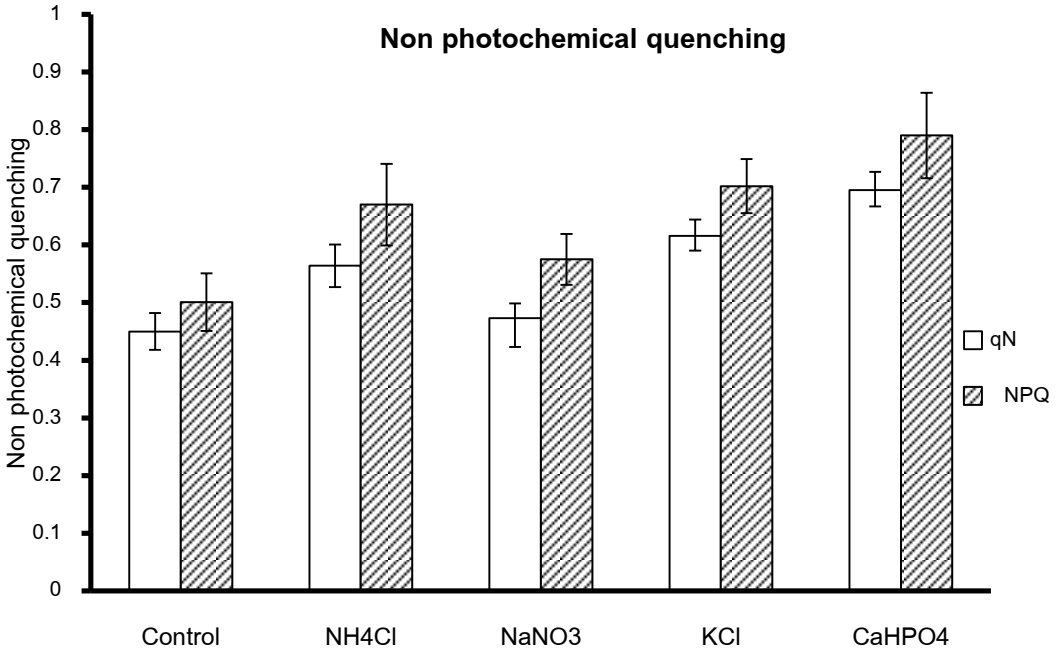

Figure 3.15 Non photochemical quenching of seedling oil palm with over supply of nutrients compared to the control. Controls were seedlings in pots of soil. qN and NPQ components are shown as means ± 95% confidence limits.

In all cases, the effects of nutrient addition on qN and NPQ were similar. NPQ was higher than qN. The pattern of nutrient-input seedling depicted by Non-photochemical quenching is the reverse from the previous parameters. All additions of nutrients treatments on the seedlings had a higher qN and NPQ than the control except for the addition of nitrate where there was no significant effect on qN or NPQ, Furthermore, phosphate-fed seedling showed the highest on both components, followed by ammonium, potassium and nitrate-fed seedlings.

Nutrient uptake summary

Summary of short term effects of addition of oversupplies of major plant nutrient to seedling Oil Palm.

The effects of the following key plant nutrients on PAM parameters were measured:

ammonium (NH_4^+)

nitrate (NO_3^-)

phosphate (PO_4^{3-})

sodium (Na^+)

potassium (K^+)

Additional nutrient in the seedling oil palm plant has significant effects on ETR rate compared to the control. However, the effect is a decrease in ETR not an increase as might be expected from the standard ideas in plant physiology (Atwell, *et al*., 1999). Addition of phosphate had the least effect on PAM parameters apart from qN and NPQ. This suggests that the plants already had sufficient P (Phosphate) in storage in the plant as polyphosphate.

Plants depend upon K (Potassium) to regulate the opening and closing of stomates the poorest through which leaves exchange carbon dioxide (CO_2), water vapor, and oxygen (O_2) with the atmosphere. K is also necessary for osmoregulation and cell function. K is a cation in solution and so binds to clay and organic material in soils.

Plants fed nitrate has a similar yield (Y) compared with control but significantly different yields were found with ammonia (NH_4^+) and P. Added nitrate to Oil Palm seedlings has similar maximum Gross photosynthesis (Pg_{max}) compared with control but significant decreases in Pg_{max} were found in the case of added K chloride.

Seedling plants with additional nitrate had similar Optimum irradiance (E_{Opt}) compared with control but significant differences in Optimum irradiance were found with potassium chloride, ammonium chloride and phosphate. Overall $NaNO_3$ had the least effect of all the added nutrients.

The non - photochemical quenching parameters, qN and NPQ are usually thought to be indicators of stress in plants (Baker, 2008). The results from the addition of ammonia, nitrate, potassium and phosphate are not easy to interpret. Phosphate addition increased qN and NPQ the most compared to the controls. Addition of ammonia and potassium resulted in smaller but statistically significant increases in qN and NPQ. Nitrate had no apparent effect on either qN or NPQ.

CHAPTER 4

Discussion

4.1 Photosynthesis

Oil Palm is known to be a C3 plant, but oil palm has high photosynthesis rates and photosynthetic efficiencies when compared to other C3 plants such as rice. Photosynthesis saturates at high irradiances and so Oil Palm is a classic "sun" plant, a characteristic usually thought to be more characteristic of C4 plants (Atwell, *et al*., 1999). C3 plants usually exhibit lower rates of photosynthesis due to photorespiration, sensitivity to O_2 concentration, light saturation and the high CO_2 compensation points when compared to plants with a C4 photosynthesis rate (Dufrene and Saugier, 1993; Ibrahim, *et al*., 2010; Jaafar and Ibrahim, 2012). Adult Oil Palm exhibits high photosynthetic rates and higher photosynthetic efficiencies during the middle of the day showing that it is a sun plant able to accommodate full sunlight with minimal photoinibition. This study found that the oil palm plant has a high photosynthesis rate, which similar to the conclusions drawn from the IRGA-based study by Dufrene and Saugier (1993). However, variation in light - saturated photosynthetic rate as 22-24 µmol m^{-2} s^{-1} can be found in oil palm from different sites (Henson and Chai, 1998).

Oil Palm seedlings need less PPFD irradiance compared to adult and juvenile Oil Palm (about 340 vs 600 µmol quanta m^{-2} s^{-1}) and produce a slightly higher maximum yield (Table 3.4 and see Jaafar and Ibrahim, 2012). The optimum irradiance values (E_{opt}) in seedling, juvenile and adult Oil Palms are typical of sun plants with the significant photo-inhibition at high irradiances (Jaafar and Ibrahim, 2012). The estimate of the maximum optimum irradiance for adult plants about 816.8 ± 45.64 µmol photon m^{-2} s^{-1} in the present study also agrees with the conclusions drawn using IRGA methods by Dufrene and Saugier (1993). Oil Palm has a high photosynthetic efficiency (α_0) on Chl *a* basis about 0.918 ± 0.024 m^2 g Chl a^{-1} and it is notable that the photosynthetic efficiency is higher in adult plants than in juvenile plants on both a surface area basis and chlorophyll bases (Table 3.2).

Maximum gross photosynthesis (Pg_{max}) estimated using PAM methods in the present study in adult plants (126.1 ± 3.14 µmol O_2 m^{-2} s^{-1}) is closely comparable to the results found for Pg by Dufrene and Saugier (1993). The optimum irradiance for adult plants found in the present study are in agreement with Dufrene and Saugier (1993) and Jaafar and Ibrahim (2012) however, Dufrene and Saugier (1993) using IRGA methods did not observe the significant photoinhibition found in the present study using PAM methods in adult plants (Figures 3.2 and 3.4). Ritchie (2012) studied photosynthesis in the blue water lily (*Nymphaea caerulea*) at Phuket and reported that maximum gross photosynthesis (Pg_{max}) during daytime was ≈ 0.5 – 0.6 gC m^{-2} h^{-1} which is almost equal to that in Oil Palm. Water lily can be used as an example of a C3 plant adapted to very high irradiances. In the original paper, the electron transport rates of water lily were reported as rETR because experimental measurements of absorptance were not available at the time. Subsequently Abt_{465nm} of water lily leaves was measured using the newly developed RAT machine (98.2 ± 0.19, n = 40, Ritchie and Runcie, 2014). Converting rETR into ETR, water lily has an ETR_{max} of 67.04 ± 1.51 µmol e^- m^{-2} s^{-1} and hence the carbon fixation rates were about 0.6 to 0.7 g C m^{-2} h^{-1} or about 17% higher than originally reported. This estimate of ETR_{max} of the water lily is similar to Oil Palm and optimum irradiance (E_{opt}) in water lily as 920 ± 39 µmol quanta m^{-2} s^{-1} PPFD is much higher in value compared to optimum irradiances of about 350 and 600 µmol photons m^{-2} s^{-1} PPFD in Oil Palm juveniles and adults (Table 3.2).

Gross photosynthesis (Pg) of juvenile oil palm plants on a chlorophyll basis was surprisingly low, about 89.21 ± 6.25 µmol O_2 mg Chl a^{-1} h^{-1} (Table 3.2). Maximum Gross photosynthesis (Pg_{max}) in adult Oil Palm was much higher, about 305.3 ± 10.73 µmol O_2 mg Chl a^{-1} h^{-1} which is higher than the absorptance corrected Pg_{max} value in water lily (264 ± 9.2 µmol O_2 mg Chl a^{-1} h^{-1}). The very high photosynthetic rate of adult leaves of Oil Palm is despite their high chlorophyll content on a surface area basis. Photosynthetic efficiency (α_0) in blue water lily (Ritchie, 2012) was only 0.191 ± 0.0092, which was considered a rather low efficiency on a surface area basis. Ritchie and Bunthawin (2010b) showed that the Phuket pineapple variety has higher photosynthetic parameters compared to Oil Palm (Table 3.2); maximum yield was about 0.7 in the day time, ETR_{max} was 76.3 ± 2.64 µmol e^- m^{-2} s^{-1} ($Abt_{465\ nm}$ corrected value from Ritchie and Runcie 2014) on a surface area basis and optimum irradiance was 755 ± 42 µmol photons m^{-2} s^{-1} PAR. Phuket pineapple (Ritchie and Bunthawin, 2010a) which is a CAM plant has a high photosynthetic efficiency (α_0) when calculated on a surface area basis (0.27 ± 0.01): essentially the same as found in Oil Palm (0.272 ± 0.018). Adult Oil Palms have an exceptionally high photosynthetic efficiency compared to most vascular plants (Table 3.2). The asymptotic photosynthetic efficiency (α_0)

calculated at zero irradiance is a very esoteric measurement. It is not the efficiency of photosynthesis under realistic conditions. Perhaps more informative is the calculation of the photosynthetic efficiency at an optimum irradiance (α_{Eopt}) (Table 3.2). The photosynthetic efficiency of Oil Palm at an optimum irradiance (α_{Eopt}) is 0.0750 ± 0.0125 or about 7.5% for juveniles and 0.1000 ± 0.00662 or about 10% of adult plants. These are high values for photosynthetic efficiency under realistic irradiances. Dufrene and Saugier (1993) calculated net photosynthetic efficiencies for adult Oil palms to be about 5.1%, which allowing for respiration, is consistent with our estimates of the gross photosynthetic efficiency. The optimum irradiance of adult oil palms (E_{opt}) is rather low compared to orchids, pineapples and water lily (Ritchie, 2012; Ritchie and Bunthawin, 2010a, 2010b) making Oil Palm less able to exploit full sunlight than orchids, pineapples and water lilies because of higher levels of photoinhibition under full sunlight.

Non-photochemical quenching parameters measure how much energy is lost as low grade heat from the photosynthetic apparatus and are a function of the proton motive force gradient across the thylakoid membranes of the chloroplasts (Genty, *et al*., 1989; Schreiber, *et al*., 1995; Rascher, *et al*., 2000; Baker, 2008). Non-photochemical quenching (NPQ) in adult Oil Palm is about 50% higher than qN under high PPFD irradiance in both juvenile and adult Oil Palms. The qN and NPQ values in adult plants are higher than found in juvenile plants. The maximum qN and NPQ values in Oil Palm (Table 3.2) are comparable to those found in most vascular plants (Ritchie, 2012; Ritchie and Bunthawin, 2010a, 2010b).

Oil Palm juveniles have the PPFD irradiance E_{opt} of about 340 µmol quanta m^{-2} s^{-1} which is less than in adult Oil Palm where E_{opt} is about 600 µmol quanta m^{-2} s^{-1}. Adult plants also have a slightly higher maximum yield. Oil Palm juveniles and adults both have a higher photosynthetic efficiency than seedling plants. Gross photosynthesis of juvenile oil palm plants on a chlorophyll basis was low, about 89.21 ± 6.25 µmol O_2 mg Chl a^{-1} h^{-1} and maximum Gross photosynthesis (Pg_{max}) in adult Oil Palm was about 305.27 ± 10.73 µmol O_2 mg Chl a^{-1} h^{-1}. The photosynthetic efficiency of Oil Palm at an optimum irradiance (α_{Eopt}) is 0.0750 ± 0.0125 or about 7.5% for juveniles and 0.1000 ± 0.00662 or about 10% of adult plants. Non-photochemical quenching (NPQ) in adult Oil Palm is about 50% higher than qN under high PPFD irradiance in both juvenile and adult Oil Palms. The qN and NPQ values in adult plants are higher than found in juvenile plants but qN and NPQ values found in Oil Palm were generally lower than found in orchids, pineapples and water lilies (Ritchie and Bunthawin, 2010a, 2010b; Ritchie, 2012). This shows that the photosynthetic mechanism of Oil Palm is in general not severely damaged by high irradiance.

Oil Palm plants show diurnal effects on photosynthesis (Figures 3.9 and 3.10, Suppl. Tables 3.1, 3.2 and 3.3 and see Apichatmeta and Ritchie, 2016 and Apichatmeta, *et al*., 2016, submitted MS). Diurnal effects on photosynthesis as measured using PAM machine have been previously reported in orchids, pineapples and water lilies (Ritchie and Bunthawin, 2010a, 2010b; Ritchie, 2012) but does not occur in the lichen *Dirinaria pinctata* (Ritchie, 2014). It is notable that not only does the maximum rate of photosynthesis (Pg_{max}) vary over the course of daylight (Figure 3.10) but the *shape* of the P vs E curve changes over the course of the day as indicated by shifts in optimum irradiance (E_{opt}) (Figure 3.9). Changes in the shape of Pg vs irradiance curves no doubt commonly occurs in plants, but its importance is generally not noted, but are only noticeable if both E_{opt} and Pg_{max} are measured using a large number of replicates and can easily not be noticed in light curves (Apichatmeta and Ritchie, 2016; Apichatmeta, *et al*., 2016, submitted MS). Photosynthetic efficiency (α_0) is directly proportional to the ratio Pg_{max}/E_{opt}. Interestingly, the diurnal effects on Pg_{max} and E_{opt} have the combined effect that seedling, juvenile and adult plants not only have similar photosynthetic efficiencies when calculated on a chlorophyll basis (Suppl. Tables 3.1, 3.2 and 3.3) but the maximum efficiency are found in all three at about midday ($\alpha_0 \approx 1.03 \pm 0.032$ m^2 g Chl a^{-1}). On a surface area basis α_0 is in the order seedling < juvenile < adult, but this largely reflects the difference in leaf morphology and hence Chl *a* per unit leaf area. Photosynthesis in Oil Palm is optimized for very high irradiances with high photosynthetic efficiencies at high irradiances with minimized photoinhibition. Thus, Oil Palm is not only capable of high photosynthetic rates, but is able to change the shape of its P vs E curves in such a way as to optimize efficient use of high irradiance not only in adult plants but juvenile/seedling plants as well. Diurnal curves show that the plant adjusts the *shape* of its P vs I curves over the course of a day. This is a great advantage in a “sun” plant.

4.2 Nutrient uptake

It is important to know the nutrition status of a crop plant. Oversupply of nutrients or imbalance between nutrients also reduces the efficiency of nutrient use (Ninnon, *et al*., 2010). In addition, an insufficient use of nutrients leads to land degradation. Biological nitrogen fixation and manure recycling are key local nutrient sources which are not always optimally exploited. The inability to match crop harvests with a sufficient nutrient return leads to depletion of nutrients and organic matter, reducing soil quality and increasing the risk of land degradation through erosion and of agricultural incursion into virgin ecosystems. Some other studies have attempted to use PAM methods to look at the effects of added nutrients on plants. Mangrove plants fertilized with P value had did not exhibit in the vigorous growth responses by observed in the N. Enrichment with the P alone had no effect by P concentration and N:P ratios in roots or shoots of mangrove plants, but when applied together with N, P concentrations in shoots increased growth by about 29% and N:P ratios in roots and shoots increased about 68% and 91%, respectively (Naidoo, 2009). Shortages of water and other nutrients such as sulphur, zinc, selenium, etc. can limit N and P use efficiency, preventing the best use being made of these major nutrients (Elanchezhian, *et al*., 2014). Nutrient enrichment of mangrove plants in freshwater did not affect photosynthesis of mangrove seedlings (Mangora, 2016). Cheeseman, *et al*. (1991) was unsuccessful in using PAM methods to detect nutrient deficiencies in mangroves. MacFarlane (2003) was successful in detecting zinc toxicity in mangroves using PAM methods. In other studies chlorine toxicity could be measured easily in the green alga *Chlorella* (Saetae, *et al*., 2013) and arsenic toxicity in the freshwater aquatic, *Wolffia* (Ritchie and Mekjinda, 2014, 2016) using PAM methods.

The presence of Na in the environment and its uptake by plants can reduce the amount of K required to meet the plants basic metabolic requirements. Thus, in the presence of Na, the critical level of K can be reduced for example, the lowest tissue K level at which 95% of the maximum yield of field vegetable crops can be achieved (Greenwood and Stone, 1998).

High nutrient supply (HNS) appears to aid the green alga *Ulva lactuca* in resisting short-term stress caused by combinations of high PAR and UVR and increased temperature. For *Ulva lactuca*, Figueroa, *et al*. (2009) were able to show that both the pigment content and the maximum level of photosynthesis increased under HNS; thus, a

similar increase in the number of reaction centers of PSII and PSI would be expected to be found.

Excess phosphorus remains in soil and combines with micronutrients, which convert it into unutilized (insoluble) form and can lead to the expression of micronutrient deficiency symptoms. High phosphorus can lead to deficiencies of zinc, ferrate and manganese. Plants have low efficiency of utilization of those elements and so the addition of micronutrients might not be effective. Excess nitrogen can also cause negative effects causes plants produce too many leaves and flowering is slowed or the plants do not flower. Excess potassium can also act as an inhibitor to reduce effective utilization of magnesium and calcium by plants. A potassium fertilizer with magnesium and calcium addition is recommended for Oil Palm (Office of the Cane and Sugar Board**,** 2011).

PAM machines measure the light reactions of photosynthesis and are capable of collecting large amounts of data very quickly. They are commonly used in studies of physiological stress and so it was logical to try using PAM methods to detect short term responses of Oil Palm plants to possible nutrient deficiencies. If a plant was limited by a nutrient such as N-sources, potassium or phosphate it was expected that an increase in photosynthesis would be observable if the photosynthesis of the plant was limited by the nutrient supplied to it. Juvenile plants growing in standard potting mix were used. Plants were offered NH_4Cl, $NaNO_3$, KCl and $CaHPO_4$ at 10 mol m^{-3} for 3 hours and rapid light curves performed on the plants after 3 hours and compared to the controls. The results (Chapter 3) were unexpected. All the plants offered extra nutrients showed consistent inhibition of photosynthesis to varying degrees. Longer term experiments are probably needed to give the plants the time to respond to added nutrient supply but the experiments do show that using PAM to quickly test for nutrient deficiencies in an Oil Palm nursery situation is not straightforward.

CHAPTER 5

Conclusion

5.1 Photosynthesis

Photosynthetic efficiency in oil palms is highest in adult with lower efficiencies are found in juveniles and seedlings, respectively, and is positively related to the leaf surface area and chlorophyll content in the leaves. The chlorophyll content in adult leaf is 474.01±43.52 mg/m^2. The photosynthetic efficiency is a function of both electron transport rate (ETR) and Optimum irradiance. However, each leaf stage favors different optimum irradiance, and this leads on to effects on the efficiency of photosynthesis. The seedling has the highest efficiency in the morning (9:00) and decreases during the course of the day. Adult plants need more light and their photosynthetic efficiency is highest in the afternoon (15:00). In juvenile and adult plants E_{opt}, Pg_{max} and α_0 tend to maximize about midday. In seedlings, E_{opt} and Pg_{max} are highest in the early morning but maximum α_0 is at about midday. Oil Palms strategically optimize photosynthesis for high irradiances.

5.2 Nutrient uptake effects on photosynthesis

The nutrient addition experiments were conducted to study the short-term effect of addition of common plant nutrients on the photosynthetic parameters of seedlings. In the short-term experiments in the present study, there was a decline of all measured parameters after adding nutrients (NH_4Cl, $NaNO_3$, KCl and $CaHPO_4$). All of these nutrients were expected to have a stimulatory effect, but the contrary was found in the short-term experiments conducted in the present study. It is possible that positive effects would have been observed in longer term studies for example watering with different nutrients and measuring photosynthetic responses 3 or 4 days later. It is also possible that photosynthesis in the plants used in the experiments were not rated-limited by any of the nutrients tested.

Short-term incubations where plants are offered a nutrient and a photosynthetic response measured shortly afterwards are not an appropriate protocol to detect nutrient deficiencies in Oil Palm. Long term experiments over several days are probably needed. It is concluded that the use of PAM techniques to monitor nutrients deficiencies needs to be done using more carefully designed experiments and short-term effects are not necessarily apparent using PAM or might be difficult to interpret. It is possible that the decrease in ETR observed in response to added N-sources, KCl or $CaHPO_4$ might reflect a diversion of $NADPH_2$ and ATP produced from the light reactions away to driving ion transport rather than directed to the Calvin Cycle but this would have to be the subject of a much more extensive study (Clarkson and Hanson, 1980; Atwell, *et al*., 1999; Baker, 2008; Macfarlane, 2003).

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Appendix

*** = significant**

ns = nonsignificant and Difference value

used to identify significant differences between Control, NH_4Cl, $NaNO_3$, KCl and $CaHPO_4$ treatments in oil palm plants.

Parameters	Control	NH_4Cl	$NaNO_3$	KCl	$CaHPO_4$
Yiel (Y)	0.704±0.012	0.66±0.01	0.688±0.011	0.672±0.009	0.658±0.013

Parameter	Tukey Test Value ± 0.021
Control, NH_4Cl	0.44
Control ,$NaNO_3$	0.78
Control, KCl	0.58
Control, $CaHPO_4$	0.42
NH_4Cl,$NaNO_3$	0.0004
NH_4Cl, KCl	0.067
NH_4Cl, $CaHPO_4$	0.797
$NaNO_3$, KCl	0.017
$NaNO_3$, $CaHPO_4$	0.0005
KCl, $CaHPO_4$	0.069

Multiple Comparisons	NH_4Cl	$NaNO_3$	KCl	$CaHPO_4$
Control	*	ns	*	*
NH_4Cl	ns	*	ns	ns
$NaNO_3$	*	ns	ns	*
KCl	ns	ns	ns	ns
$CaHPO_4$	ns	*	ns	ns

Electron transport rate (ETR)

Parameters	Control	NH_4Cl	$NaNO_3$	KCl	$CaHPO_4$
ETR (µmol m^{-2} s^{-1})	25.41±1.162	14.00±0.996	17.07±0.588	15.04±0.617	14.08± 0.735

Parameter	Tukey Test Value ± 1.59
Control, NH_4Cl	3.7E-16
Control ,$NaNO_3$	2.1E-14
Control, KCl	8.2E-17
Control, $CaHPO_4$	2.5E-17
NH_4Cl,$NaNO_3$	3.6E-06
NH_4Cl, KCl	0.068
NH_4Cl, $CaHPO_4$	0.883
$NaNO_3$, KCl	1.9E-05
$NaNO_3$, $CaHPO_4$	1.7E-07
KCl, $CaHPO_4$	0.0423

Multiple Comparisons	NH_4Cl	$NaNO_3$	KCl	$CaHPO_4$
Control	*	*	*	*
NH_4Cl	ns	*	ns	ns
$NaNO_3$	*	ns	*	*
KCl	ns	*	ns	ns
$CaHPO_4$	ns	*	ns	ns

Gross Photosynthesis (Pg)

Parameters	Control	NH_4Cl	$NaNO_3$	KCl	$CaHPO_4$
Pg (µmol O_2 mg Chl a^{-1} h^{-1})	148.1±6.771	87.40± 5.802	108.3±3.425	87.05±3.595	83.64±4.284

Parameter	Tukey Test Value ± 9.328
Control, NH_4Cl	4.2E-15
Control ,$NaNO_3$	3.3E-12
Control, KCl	6.3E-17
Control, $CaHPO_4$	4.8E-17
NH_4Cl,$NaNO_3$	2.5E-07
NH_4Cl, KCl	0.915
NH_4Cl, $CaHPO_4$	0.250
$NaNO_3$, KCl	0.607
$NaNO_3$, $CaHPO_4$	0.550
KCl, $CaHPO_4$	0.934

Multiple Comparisons	NH_4Cl	$NaNO_3$	KCl	$CaHPO_4$
Control	*	*	*	*
NH_4Cl	ns	*	ns	ns
$NaNO_3$	*	ns	*	*
KCl	ns	*	ns	ns
$CaHPO_4$	ns	*	ns	ns

Photosynthetic Efficiency on Surface Area basis

Parameters	Control	NH_4Cl	$NaNO_3$	KCl	$CaHPO_4$

Photosynthetic Efficiency (α_0) Surface Area basis	0.183±0.016	0.139±0.017	0.154±0.011	0.140±0.010	0.124±0.012

Parameter	**Tukey Test Value ± 0.025**
Control, NH_4Cl	0.0004
Control ,$NaNO_3$	0.0034
Control, KCl	3.5E-05
Control, $CaHPO_4$	6.3E-07
NH_4Cl,$NaNO_3$	0.125
NH_4Cl, KCl	0.915
NH_4Cl, $CaHPO_4$	0.135
$NaNO_3$, KCl	0.054
$NaNO_3$, $CaHPO_4$	0.0005
KCl, $CaHPO_4$	0.045

Multiple Comparisons	**NH_4Cl**	**$NaNO_3$**	**KCl**	**$CaHPO_4$**
Control	*	*	*	*
NH_4Cl	ns	ns	ns	ns
$NaNO_3$	ns	ns	ns	*
KCl	ns	ns	ns	ns
$CaHPO_4$	ns	*	ns	ns

Photosynthetic Efficiency (α_0) on Chl *a* basis

Parameters	**Control**	**NH_4Cl**	**$NaNO_3$**	**KCl**	**$CaHPO_4$**
Photosynthetic Efficiency (α_0)					

Chl *a* basis (m^2 gChl a^{-1})	1.069±0.094	0.812±0.097	0.983±0.067	0.814±0.06	0.758±0.071

Parameter	**Tukey Test Value ± 0.148**
Control, NH_4Cl	0.0003
Control ,$NaNO_3$	0.123
Control, KCl	3.3E-05
Control, $CaHPO_4$	4E-06
NH_4Cl,$NaNO_3$	0.004
NH_4Cl, KCl	0.970
NH_4Cl, $CaHPO_4$	0.346
$NaNO_3$, KCl	0.0004
$NaNO_3$, $CaHPO_4$	3E-05
KCl, $CaHPO_4$	0.209

Multiple Comparisons	**NH_4Cl**	**$NaNO_3$**	**KCl**	**$CaHPO_4$**
Control	ns	ns	ns	ns
NH_4Cl	ns	ns	ns	ns
$NaNO_3$	ns	ns	ns	ns
KCl	ns	ns	ns	ns
$CaHPO_4$	ns	ns	ns	ns

Non photochemical quenching on qN

Parameters	Control	NH_4Cl	$NaNO_3$	KCl	$CaHPO_4$
Non photochemical quenching qN	0.450±0.032	0.564±0.037	0.473±0.0255	0.616±0.028	0.695±0.032

Parameter	Tukey Test Value ± 0.0584
Control, NH_4Cl	2.6E-05
Control ,$NaNO_3$	0.240
Control, KCl	2.7E-09
Control, $CaHPO_4$	1.5E-12
NH_4Cl,$NaNO_3$	0.0002
NH_4Cl, KCl	0.0234
NH_4Cl, $CaHPO_4$	3.2E-06
$NaNO_3$, KCl	5.5E-09
$NaNO_3$, $CaHPO_4$	1.4E-12
KCl, $CaHPO_4$	0.0004

Multiple Comparisons	NH_4Cl	$NaNO_3$	KCl	$CaHPO_4$
Control	*	ns	*	*
NH_4Cl	ns	*	ns	*
$NaNO_3$	*	ns	*	*
KCl	ns	*	ns	*
$CaHPO_4$	*	*	*	ns

Non photochemical quenching on NPQ

Parameters	Control	NH_4Cl	$NaNO_3$	KCl	$CaHPO_4$
Non photochemical quenching NPQ	0.501±0.05	0.67±0.071	0.575±0.044	0.702±0.047	0.79±0.074

Parameter	Tukey Test Value ± 0.110
Control, NH_4Cl	0.0003
Control ,$NaNO_3$	0.025
Control, KCl	7.1E-07
Control, $CaHPO_4$	1.2E-07
NH_4Cl,$NaNO_3$	0.0216
NH_4Cl, KCl	0.421
NH_4Cl, $CaHPO_4$	0.018
$NaNO_3$, KCl	0.0002
$NaNO_3$, $CaHPO_4$	9.4E-06
KCl, $CaHPO_4$	0.041

Multiple Comparisons	NH_4Cl	$NaNO_3$	KCl	$CaHPO_4$
Control	*	ns	*	*
NH_4Cl	ns	ns	ns	*
$NaNO_3$	ns	ns	*	*
KCl	ns	*	ns	ns
$CaHPO_4$	*	*	ns	ns