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Growth, Carbohydrate Assimilation and Leaf Gas Exchange Characteristics of *Elaeis guineensis* Jacq Seedlings under Nitrogen Fertilization

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Authors' contributions

This work was carried out in collaboration between all authors. Authors MHI, RN and PEMW designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors TSN and AR managed the analyses of the study. Author NAMZ managed the literature searches. All authors read and approved the final manuscript.

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Short Communication

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ABSTRACT

Aims: This study was conducted to characterise the growth, carbohydrate and leaf gas exchange patterns of oil palm seedlings performance (Deli Yangambi) under nitrogen fertilisation.
Study Design: The palms were fertilised with three nitrogen rates: (1) Control (90 g/palm); (2) twice the control (180 g/palm), and (3) thrice the control (270 g/palm). Randomized complete block design (RCBD) with three replications was used during the experiment using Deli Yangambi seedlings
Place and Duration of Study: Ladang 2, Universiti Putra Malaysia from March 2017 to June 2017.
Methodology: The nitrogen treatments started when the oil palm seedlings reached four months old using Urea (46% N) as a source of nitrogen. Growth, carbohydrate and leaf gas exchange

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properties were measured at the end of the treatment period of two months.
Results: As the level of nitrogen application increased, from 90 - 270 g/palm, the plant height, number of frond per plant, basal diameter, leaf area per seedling, root biomass, total dry matter, net assimilation rate (NAR) and relative growth rate (RGR) was statistically significantly increased between nitrogen treatments. The production of total non-structural carbohydrate (TNC) was reduced under high rates of nitrogen. The reduced in TNC under high nitrogen rates was supported by a decrease in sucrose and starch content under low nitrogen fertilisation. The net photosynthesis (A), stomatal conductance (gs), transpiration (E) and Intercellular CO2 also was found to be increased under high nitrogen rates indicated that higher rates of nitrogen would enhance the leaf gas exchange characteristics of the palms.
Conclusion: In conclusion, the growth of oil palm seedlings growth was enhanced with high rates of nitrogen supplementation. As the nitrogen rates (90>270 g/palm), more production of TNC was produced and increased the sink strength of the palms. The enhanced growth and TNC accumulation also increased the leaf gas exchange properties that were showed by increased in A, gs, E, Ci and Water use efficiency in the oil palm seedlings.

Keywords: Deli Yangambi palms; nitrogen uptake; plant development; carbohydrate; leaf gas exchange.

1. INTRODUCTION

The oil palm (*Elaeis guineensis* Jacq.) is a perennial monocotyledonous plant which belongs to the family *Arecaceae* originating from West Africa, the fruit pulp and nut that provide palm and kernel oil, respectively, made oil palm a high yielding oil-producing crop [1]. At present, palm oil production is second only to that of soybean oil regarding world vegetable oil production and the demand for palm oil is expected to increase in future [2,3]. Oil palm is an important source of oils and fats since its price is more affordable and is capable of producing about four folds of oil yield/hectare compared to other oil crops such as soybean, sunflower and rapeseed [4]. Several factors contribute to high yield of oil palm such as good agronomic practices, adequate nutrient supply, planting material and climate. Among the factors, adequate nutrient supply especially Nitrogen (N) is the most important in producing high yield oil palm [5].

Nitrogen is a component of many essential organic compounds such as amino acid, proteins and nucleic acids that play essential roles in many physiological processes including photosynthesis, respiration and transpiration [6]. The N application has been reported to influence the palm height, chloroplast development, leaf area index (LAI), susceptibility to pest and disease and consequently, bunch and oil quality planting robust oil palm seedlings is a prerequisite for the establishment of a successful planting with early fruiting and high yields at maturity [7]. Vigorous seedlings require the provision of adequate moisture and plant nutrients, chiefly N, at the nursery stage. N deficiency severely restricts growth; therefore, an effective fertilisation regime should be implemented to satisfy plant requirements [8]. Generally, N deficiency was shown to cause stunted palms and yellowing of leaves. Necrosis results if the deficiency is severe. Inadequate N supply also may delay crop production for up to 36 months. In contrast, excessive N application not only causes economic loss and environment pollution but also can result in a reduction of bunch yield [9].

In general, nitrogen is required by plants in largest amount, being crucial in both growth and plants development [10]. An increase in nitrogen usually results in an increase in crop growth and its development. According to [11], metabolic processes, based on protein, leading to increasing in vegetative and reproductive growth and the crop yield is totally dependent upon an adequate supply of nitrogen. This is because nitrogen helps to speed up all protein-based metabolic processes. Occasionally, any nitrogen removal would result in decreasing of leaf production, individual leaf area and total leaf area [12] which affect the light interception, photosynthesis process and finally the plants growth. Nitrogen plays a vital role in photosynthesis and essential for enzymatic processes. An increase in nitrogen will result in higher leaf nitrogen content [9]. Appropriate nitrogen supply was reported by many researches to have increased the leaf chlorophyll content, Rubisco activity in plants and improve photosynthetic efficiency [13-15]. Previous research also have showed that more carbohydrates can be accumulated with high levels of nitrogen supplied to the plant. The more nitrogen invested would incur more sink strength to the plant [16,17].

Due to the growing demand of palm oil, a lot of methods had been used to increase the yield of palms [1,4,5]. One of the most common methods employ among the farmers is through the application of the fertilisers. Nitrogen is known to be the most abundant nutrients in plants and often plays an important role in quality of crops [18,19]. Plants absorb nitrogen either as nitrate ion (NO_3^-) or as ammonium ion (NH_4^+). The sources of nitrogen commonly used for crop production mainly ammonium nitrate and urea. Previously, there were many studies have been conducted on nitrogen fertilization input on oil palm seedlings. However, the previous study was more focused on the impact of compound fertilization on the growth and agronomical characteristics of this particular plants. There were lack and a few studies on impact of single fertilizer especially Urea. The carbohydrate and leaf gas exchange responses of Oil palm seedlings to nitrogen was scarcely documented. With that, there is a need to conduct experiments on the input of nitrogen on the growth, leaf gas exchange and carbohydrate accumulation of oil palm seedlings. With that the main objective of the experiment was to investigate the effects of different rate of Urea on the growth, leaf gas exchange and carbohydrate accumulation in Deli Yangambi seedlings.

2. MATERIALS AND METHODS

2.1 Experiment Place and Treatments

The experiment was carried out in Ladang Dua of the Faculty of Agriculture, Universiti Putra Malaysia. The 3-month-old tenera (Deli Yangambi; From Felda Agricultural) oil palm were left for 1 month to acclimatize in a nursery until ready for the treatments. Four-month-old oil palm seedlings were transplanted into black polythene bags containing 20 kg soil. Topsoil (0-30 cm) of the Rengam series collected from Taman Pertanian Universiti was used. The nitrogen source used in the study was urea (46% N) and there were three rates applied i.e. 90 g/palm (control according to the field handbook for oil palm nursery management (Rankine and Fairhurst [20]), 2X control (180 g/palm) and 3X control (270 g/palm). These were applied three times for two months (30% one weeks after planting; 30% four weeks after planting and 40% six weeks after planting).

2.2 Plant Growth Measurements

Total plant biomass was taken by calculating the dry weight of root, boles and leaves per seedling. Destructive plant analysis was carried out at 8 weeks after treatments. The plant parts were placed in paper bags and oven dried at 80ºC until constant weight was reached using electronic weighing scale (CDS 125, Mitutoyo Inc, Japan). Leaf area per plant was measured using a leaf area meter (LI-3100, Lincoln Inc, USA). The leaves were arranged within the field of view, and overlapping of adjacent leaves was avoided. Growth analysis was calculated on an individual plant basis through the measurement of total plant leaf area and dry weight. NAR and RGR were calculated based on biomass and leaf area parameters according to the formula reported by [21].

2.3 Carbohydrate Determination

Total soluble sugar was measured spectrophotometrically using the method of [22]. Samples (0.5 g) were placed in 15 mL conical tubes, and distilled water added to make up the volume to 10 mL. The mixture was then vortexed and later incubated for 10 min. Anthrone reagent was prepared using anthrone (Sigma Aldrich, St Louis, MO, USA, 0.1 g) that was dissolved in 95% sulphuric acid (Fisher Scientific, USA 50 mL). Sucrose was used as a standard stock solution to prepare a standard curve for the quantification of sucrose in the sample. The mixed sample of ground dry sample and distilled water was centrifuged at a speed of 3400 rpm for 10 min and then filtered to get the supernatant. A sample (4 mL) was mixed with anthrone reagent (8 mL) and then placed in a water-bath set at 100ºC for 5 min before the sample was measured at an absorbance of 620 nm using a spectrophotometer model UV160U (Shimadzu Scientific, Kyoto, Japan). The total soluble sugar in the sample was expressed as mg sucrose g^{-1} dry sample. Starch content was determined spectrophometrically using a method described by Thayumanavam and Sadasivam [23]. In this method, dry sample (about 0.5 g) was homogenized in hot 80% ethanol to remove the sugar. The sample was then centrifuged at 5000 rpm for 5 min and the residue retained. After that, distilled water (5.0 mL) and 52% perchloric acid (6.5 mL) were added to the residue. Then the solution was centrifuged and the supernatant separated and then filtered with Whatman No. 5 filter paper. The processes were repeated until the supernatant was made up to 100 mL. A sample (100 µL) of the supernatant was added to distilled water until the volume became 1 mL in a test tube. After that, anthrone reagent (4 mL, prepared with 95% sulphuric acid) was added to the test tube. The mixed solution was placed in the water bath at 100ºC for eight min and then cooled to room temperature, and then the sample was read at absorbance of 630 nm to determine the sample starch content. Glucose was used as a standard and starch content was expressed as mg glucose equivalent g−1 dry sample. The total non-structural carbohydrate was calculated as the sum of total soluble sugar and starch content.

2.4 Leaf Gas Exchange Measurements

Measurements were taken using a closed system, infra-red gas analyzer (LICOR 6400 Portable Photosynthesis System; LICOR Inc. Nebraska, USA) by placing the lamina of fully expanded leaves of the second frond on the terminal bifid lobes in a leaf cuvette set with optimal growth conditions. Measurements used standard optimal cuvette conditions for oil palm at 1000 µmol m^2 s^{-1} photosynthetically active radiation (PAR), 400 ppm CO_2 concentration, 30ºC leaf temperature and 60% relative humidity. Measurements of gas exchange were carried out between 1000 to 1100 after the CO_2 enrichment procedure. Net photosynthesis rate a (µmol $m\text{-}^2s^{-1}$), transpiration rate (mol $m\text{-}^2s^{-1}$), stomata conductance (mmol $m\text{-}^2s^{-1}$) and intercellular CO_2 (ppm) were simultaneously recorded. Water use efficiency was calculated by dividing net photosynthesis with transpiration rate. Data were stored in the LICOR computer within the console, and analyzed by “Photosyn Assistant “software that calculated several parameters. Several precautions were applied to avoid errors during measurement, e.g. cleaning and drying of leaf surfaces before placing them in leaf cuvette.

2.5 Statistical Analysis

Statistical Package for Social Sciences (SPSS) version 21 was used to analyze the data that have been recorded. A two-way ANOVA Test was conducted to analyze data for all the parameters in the experiment. Data are significant if the p-value level ≤ 0.05.

3. RESULTS AND DISCUSSION

3.1 Oil Palm Growth

Table 1 shows the growth characteristics of Oil palm seedlings, as affected by different nitrogen rates. Nitrogen fertilization shows a significance influence on the growth characteristics of oil palm seedlings ($P \leq 0.05$). As nitrogen increases from 90>180>270 g/ plant, the plant characteristics such as plant height, frond number, basal diameter and leaf area was found to be higher than the recommended nitrogen fertilization (90 g/plant). The 180 g (102.22 cm) and 270 g/plant (92.21 cm) seedlings produced taller palms than 90 g/plant palms which high only 88.41 cm at end of the treatment. At the age of 6 months, only 12.4 fronds were produced by 90g/plant seedlings compared to 15.6 by 180 g/plant and 14.8 by 270 g/plant treatments. The basal diameter for 180 and 270 g/palm was 44.8 cm and 45.2 cm respectively compared to only 32.4 cm for 90 g/ palm treatments. At the end of the experiment, the total leaf area for the respective 180 and 270 g/plant was 10% and 3% higher than 90 g/plant treatment. The total plant biomass was significantly lower ($P<0.05$) in 90 g/plants treatments than the higher nitrogen rates palms. The total plant biomass of 90 g/plant is 50% lower than the average total biomass of the higher nitrogen treatments. Table 1 clearly indicates that the enhancement of plant total biomass was due to enhanced growth aboveground compared to the growth belowground, due to higher aboveground biomass (leaf and bole biomass) compared to belowground biomass (root biomass). The net assimilation was also enhanced under high level of nitrogen, according to Cure and Acock [24], the increase in NAR is attributable to the increased leaf area of plants growing in sufficient nutrient especially nitrogen. According to Blackman [25] relative growth rate (RGR) is defined as the dry weight increase per unit of dry weight present per unit of time. Present result showed 1 and 2 fold increase in RGR enhanced biomass by 50% than seedlings grown at normal fertilization rates (90 g/palm). The increase in vegetative growth of oil palm seedling with increased application of nitrogen might be due increase in sink strength of the palm with increased in application of nitrogen [26]. This because nitrogen is related to sink strength of plant, the more nitrogen that invested would initiate more sinks to the palm. This justifies the increase in growth patterns of oil palm seedling with increased in nitrogen application [27].

Table 1. Plant growth characteristics of oil palm seedlings, as affected by different nitrogen rates

Plant characteristics	Nitrogen (g/ plant)		
	90	**180**	**270**
Plant Height (cm)	88.41±1.32[c]	102.14±1.56[a]	92.2±3.11[b]
Frond number	12.42±0.98[c]	15.62±1.23[a]	14.88±0.26[b]
Basal diameter (mm)	32.14±1.23[c]	44.83±2.04[a]	44.25±0.87[b]
Leaf Area (cm^2)	2920.23±0.43[c]	3219±2.56[a]	3012±0.54[b]
Total biomass (g)	91.31±0.54[c]	172.23±0.77[b]	198.03±0.10[a]
Leaf biomass (g)	30.46±0.32[c]	64.15±2.42[b]	76.52±5.21[a]
Bole biomass (g)	44.31±0.21[c]	82.42±0.21[b]	97.63±2.23[a]
Root biomass (g)	17.34±0.32[c]	25.16±0.44[b]	32.21±0.12[a]
NAR (g/cm^2/week)	0.21±0.12[c]	0.25±0.09[b]	0.35±0.12[a]
RGR (g/g/week)	1.01±0.09[c]	1.12±0.01[b]	1.22±0.13[a]

All analyses are mean ± standard error of mean (SEM). N = 15. Means not sharing a common alphabet were significantly different at P ≤ 0.05 using Duncan Multiple Range Test (DNMRT)

3.2 Carbohydrate profiling

Fig. 1 shows the carbohydrate profiling of oil palm seedlings as affected by nitrogen fertilization rates. It was found that the soluble sugar, starch and total non structural carbohydrate of oil palm seedling was influenced by the nitrogen treatments (P≤0.05). At the end of the experiment, the TNC was highest under 270 g/palm (143.2 mg/g dry weight) followed by 180 g/palm (121.7 mg/g dry weight) and lowest under control (90 g/palm) that recorded 101.2 mg/g dry weight. It is noted that the starch levels were found to be higher than the sucrose content in all treatment. In oil palms seedlings, the increase in starch content was larger than the increase in sugar concentration with decreasing N fertilization [28] suggesting that the low N-fertilization was able to enhance soluble sugar and starch contents, which had simultaneously enhanced the TNC. Similar observation was demonstrated by other researchers [29,30]. The accumulation of TNC in high nitrogen-fertilized plant might be due to increase in sink size of the plant when more nitrogen is invested; hence, increasing the translocation of carbohydrates to other plant parts [31].

3.3 Leaf Gas Exchange Status

Table 2 shows the leaf gas exchange results for oil palm seedlings under different nitrogen application. It can be seen that all the leaf gas exchange was influenced by nitrogen application (P≤0.05). At the end of the experiment, palm under 90 g/plant only recorded 10.45 μmol/m^2/s

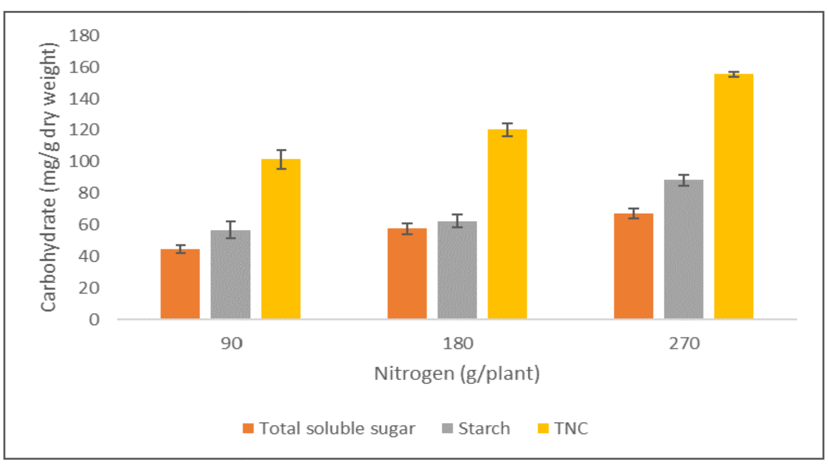

Fig. 1. The carbohydrate profiling of oil palm under different nitrogen application. Data are means ± standard error of differences between means of 15 replicate. (TNC = Total non structural carbohydrate)

Table 2. Plant leaf gas exchange characteristics of oil palm seedlings, as affected by different nitrogen rates

Plant characteristics	Nitrogen (g/ plant)		
	90	**180**	**270**
Photosynthesis, *A* (μmol $m^{-2}s^{-1}$)	10.45±1.32^c	14.14±1.56^b	16.2±3.11^a
Stomata conductance, *gs* (mol $m^{-2}s^{-1}$)	0.31±0.98^c	0.36±1.23^b	0.40±0.26^a
Transpiration rate, *E* (mol $m^{-2}s^{-1}$)	4.14±1.23^c	4.83±2.04^b	5.25±0.87^a
Intercellular CO_2, C_i (ppm)	220.23±0.43^c	231±2.56^b	240±0.54^a
Water use efficiency, WUE	2.52±0.54^c	3.23±0.77^b	3.13±0.10^a

All analyses are mean ± standard error of mean (SEM). N = 15. Means not sharing a common alphabet were significantly different at P ≤ 0.05 using Duncan Multiple Range Test (DNMRT)

photosynthesis compared to 180 and 270 g/plant that recorded 14.4 and 16.2 μmol/m^2/s photosynthesis respectively. The increase in nitrogen fertilization has subsequently resulted in higher stomatal conductance and transpiration rate in oil palm seedlings. The gs for 180 and 270 g/plants was 355 and 401 respectively compared to only 312.4 for 90 g/plant. The transpiration rate for the respective 180 and 270 g/plant was 17% and 27% higher than 90 g/plants palms. The increase in the leaf gas exchange properties of oil palm seedlings with the increase in nitrogen application might be due to increase in Rubisco activities with increased in nitrogen application to the palms. As nitrogen levels increase it will cause enhancement in mesophyll activity, chlorophyll content, Rubisco activity or amount of enzyme affecting the photosynthesis rate. The result obtained agreed with previous work carried out by [26] where they observed the leaf gas exchange characteristics of *Chrysanthemum indicum* increased with addition of nitrogen. The result shows evidently higher Ci of palm with higher rates of nitrogen. The Ci of 90 g/plants was 220.2 ppm, 180 g/plant was 231.00 ppm and 270 g/plant was 240.00 ppm. The higher Ci values with high nitrogen application indicate that more saturated CO2 inside the leaf mesophyll under high levels of nitrogen [32]. Also the Ci inside the leaves also can increase due to higher mitochondrial respiration due to stomata closure that happened under high stress levels [26]. The enhanced nitrogen fertilization also showed enhanced in WUE. At the end of the experiment the WUE of 90 g/plants was 28% and 23% less than 180 g/plant and 270 g/plant respectively. Water use efficiency is related with carbon dioxide (CO_2) assimilated by photosynthesis and water lost through transpiration [33]. Higher photosynthesis rate causes an increase in WUE while decrease in transpiration rate. Nitrogen application can affect the WUE, increasing the root surface area, depth and biomass [34-37]. On the other hand, nitrogen also enhances net assimilation rate which in turn improves the WUE. Nitrogen addition will cause more water uptake to assist in photosynthesis process and other cell metabolic activities. So this justifies why WUE was enhanced with increasing N levels in the present study. A study conducted by [27] in tobacco also in agreement with the present study. The result showed a significantly increased of net photosynthetic rate, stomatal conductance, transpiration and intercellular CO_2 of tobacco fertilised with high nitrogen rates. So it can be concluded that high levels of nitrogen can enhance the leaf gas exchange properties of oil palm seedlings that observed in the present study.

4. CONCLUSION

In this work, oil palm seedlings were treated with three rates of nitrogen fertiliser to assess the effects of nitrogen fertilisation on palm growth, leaf gas exchange and carbohydrate partitioning. It was found that the growth of oil palm seedlings was enhanced with enhanced nitrogen rates. It was found that the increase in nitrogen levels have enhanced the vegetative properties of oil palm seedlings. Under high nitrogen levels, there was an accumulation of TNC that indicate enhanced sink strength under high levels of nitrogen rates. The result obtained from the study also indicate that oil palm seedling would have enhanced the leaf gas exchange characteristics under high levels of nitrogen. So the increased in nitrogen levels have improved the growth, TNC and leaf gas exchange of oil palm seedlings in the present study.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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