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Evaluation of Nitrogen Uptake Efficiency of Different Oil Palm Genotypes Using ^{15}N Isotope Labelling Method

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ABSTRACT

High demands for palm oil world wide induce the expansion of oil palm plantations in Malaysia. Malaysian soils which are highly weathered require high nitrogen fertilizer input in order to maintain high yield output, resulting in increase in production cost as well as inducing negative impacts to the environment. It is crucial to understand the performance of different oil palm genotypes in taking up nitrogen to increase nitrogen use efficiency, minimize environmental pollution caused by leached nitrate and maximize plantation profit, while maintaining sustainable agriculture practices. ^{15}N labelling method was utilized in a greenhouse study to quantify the nitrogen up-take performance of nine oil palm genotypes at 6 and 9 months after planting. Measurements of total dry matter, total N, and percentage N derived from fertilizer (%NdFF) were carried out during the study. At 6 month old, oil palms of different genotypes did not show any difference in nitrogen uptake with and without P fertilizer applications. However, 9 months old oil palms demonstrated significant differences between the genotypes in total dry matter production and total N taken up, hence, resulting in significant differences in N derived from fertilizer among genotypes. Oil palms at 9 months old also showed significant effects in the N uptake as affected by P fertilizer application. Genotype A (14/34 x 2367/17) demonstrated significantly higher nitrogen uptake compared to other genotypes, except for genotype F (9/103 x 2318/17). Thus, the ^{15}N labelling technique could serve as a useful assessment to the nitrogen uptake abilities of oil palm genotypes.

Keywords: Oil palm, nitrogen fertilizer, nitrogen uptake, ^{15}N isotope labelling

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INTRODUCTION

High demands for palm oil world wide have made oil palm (*Elaeis quineenis*) an important plantation crop in Malaysia, which contributes to high National Gross Export (Azman *et al.*, 2004). The total oil palm area in 2009 was 4.69 million hectares, i.e. with an increase of 4.5% as compared to the previous year. Meanwhile, total export of oil palm products, consisting of palm oil, palm kernel oil, palm kernel cake, oleochemicals, biodiesel and finished products increased by 2.9% or 0.64 million tonnes to 22.40 million tonnes in 2009 from 21.76 million tonnes recorded in 2008 (Mohd. Basri, 2010). This increase has further induced the expansion of oil palm plantations in Malaysia. Thus, more lands have been converted into oil palm plantation as a result of the increases in oil palm hectarage. However, most soils planted with oil palm in Malaysia are of the order Ultisols and Oxisols, which are low in fertility status (Goh *et al.*, 2003), and planting oil palm in these soils could cause further soil degradation (Tessens & Shamshuddin, 1983). Hence, applying additional fertilizers to compensate nutrient removal from soils by plant seems to be a direct answer to this situation.

It is important to highlight that excessive applications of fertilizers, especially nitrogen, have harmful consequences on the environments. Nitrogen applied as inorganic chemical fertilizer, especially nitrates, could easily experience leaching by rainfall due to its negative charge, and as much as half of the nitrogen fertilizers applied could be loss at the end of the planting season (Sukreeyapongse *et al.*, 2001). Therefore, increasing fertilizer rates without any proper precaution will often lead to intense environmental pollution. As fertilizers are the most expensive inputs (Sabri, 2009) and the largest variable cost item in oil palm production (Goh *et al.*, 2003), leaching of N from chemical fertilizer will not only bring negative influences to the environment but also represent a significant economic loss. In 2008 alone, the industrial plantation in Malaysia consumed 1.29 million tons of N fertilizers. A study by Goh (2005) also indicated that over-application of as much as 0.25 kg ammonium nitrate/palm/year would result in an extra cost of RM117.25 million per year to the oil palm industry in Malaysia. Hence, overcoming nutrient loss from soils via increase in fertilizer application was apparently and economically unfavourable. Nevertheless, insufficient nutrient supply to meet the requirement of oil palm will result in a significant drop in the productivity of oil palm for the subsequent years (Mohd Tayeb & Tarmizi, 2001). Mohd Noor *et al.* (2005) reported that oil palm demonstrated a slow recovery of productivity, following a decrease in fertilizer application, and the slow recovery of oil palm productivity would affect the profitability in the subsequent years. Goh (2005) indicated that under-application of fertilizers might result in the loss of oil palm yields. Hence, increasing oil palm nitrogen use has efficiency become crucial in optimizing nitrogen use, reduce fertilizer cost, and avoid N fertilizer pollutions.

Oil palm growth performance can vary due to oil palm genetics (Soh, 2004; Rafii *et al.*, 2002; Kushairi *et al.*, 1999). Research workers observed that variation of oil palm genotypes contributes in producing variable genetic expression (Norziha *et al.*, 2008), such as different heights, sizes of canopy, sizes of bunches, amounts of mesocarp, kernel contents (Kushairi *et al.*, 1999), and different vigorous levels (Soh, 2004). Meanwhile, a high level of variability in yield has been observed from oil palm progenies (Norziha *et al.*, 2008; Soh, 2004). Despite all the efforts done in this area of research, the exploitation of genotypic performance and site yield potential of oil palm planting materials are still at its infancy (Goh *et al.*, 1994). Hence, in order to maintain high oil palm yields with low chemical fertilizer inputs, it is essential to screen oil palm for the genotypes that have superior nitrogen uptake abilities. Thus, the primary objective of this study was to evaluate the ability of the oil palm genotypes for nitrogen acquisition ability at the nursery stage.

MATERIALS AND METHODS

The experiment was carried out in a glass house (with an average temperature of 29°C and a relative humidity of 96%) at the Faculty of Agriculture, Universiti Putra Malaysia. The oil palm seedlings used in this study were obtained from Sime Darby Research Sendirian Berhad (Table 1). The experiment was conducted as a 9 X 2 factorial laid out in Randomized Complete Block Design (RCBD) with four replications. The treatments comprised of nine genotypes of three-month old oil palm seedlings (as shown in Table 1) and two P levels (with P, 6.70 g palm^{-1}; and without P, 0 g palm^{-1}). A total of 72 three-month old oil palm seedlings were planted in black polythene bags containing 30 kg of Serdang series soil (*Typic paleudult*). The soil chemical properties are shown in Table 2. At planting, nutrients were added according to the fertilizer programme for oil palm seedlings (Gillbanks, 2003), namely, 4.90g nitrogen, 6.70g phosphorus, 5.00 g potassium and 3.90 g magnesium per palm. Ammonium sulphate (AS), labelled with 5% atom excess (a.e) ^{15}N, was used as a source of nitrogen. This was split-applied at day 1 and day 45 after planting to minimize the leaching of nitrogen. Each application of ^{15}N labelled AS was applied in solution formed by dissolving 11.67g of 5% a.e. AS with distilled water. Phosphorus fertilizer in the form of Gafsa phosphate rock (14% P) was applied to only 36 bags of the oil palms seedlings, which were subjected to P fertilizer, while the remaining 36 bags of oil palms seedlings would rely on the available P in the soil as a source of phosphorus without any addition of P fertilizer to reflect the P fertilizer synergistic effect to N uptakes of oil palm. Potassium and magnesium were applied in the form of Muriate of Potash and Kieserite. 20g of AJIB© was applied for micronutrients. Weeding and irrigation were carried out manually for all the pots. Using the same methods, another set of experiment was conducted separately using six-month old oil palm seedlings. Both sets of oil palm seedlings were left to grow for three months to allow the uptake of nutrient and ^{15}N labelled fertilizer. At the end of three months, destructive sampling was carried out for both sets of oil palm seedlings. Plant samples were separated into rachis and leaves, which were then oven dried at 70°C until constant weight was achieved. Dry weights of the samples were measured using analytical balance. The samples were then ground to pass through 1mm sieve and analyzed for their total nitrogen and ^{15}N enrichment (IAEA, 1983). The percentage of N derived from fertilizer (%NdFF) was calculated using the following equation, based on the isotope dilution technique (IAEA, 1983):

$$\%\,\text{Ndff} = \frac{\%\text{N} - 15\text{ a.e. plant}}{\%\,\text{N} - 15\text{ a.e. fertilizer}} \times 100 \quad [1]$$

$$\begin{aligned}\text{N yield} &= \text{Dry Matter (DM) yield of plant} \times \%\ \text{N in plant}\\ &= \text{Total N uptake in plant (g plant}^{-1}) \quad [2]\end{aligned}$$

$$\text{Fertilizer N yield} = \frac{N\ yield \times \%\ Ndff}{100} \quad [3]$$

The ability of the oil palm seedlings to take up the N fertilizer was evaluated from the total N uptake derived from the labelled fertilizer (Fertilizer N Yield). The means were compared by the analysis of variance (ANOVA) using the SAS statistical software version 9.0 (SAS, 2002), and the treatment means were separated by Student-Newman-Keuls Test at 5% probability level.

TABLE 1
Genotypes of Oil Palm (obtained from Sime Darby Research, Banting)

Genotypes	Mother Palm (Dura)	Pollen Palm (Pisifera)
A	14/34 (UR X JL)	2367/17 (AV)
B	2/35 (UR X UR)	2367/17 (AV)
C	2/209 (BD X BD)	2367/17 (AV)
D	19/19 (UR X UR)	2367/17 (AV)
E	25/49 (BD x UR)	2367/17 (AV)
F	9/103 (BD X BD)	2318/17 (AV)
G	23/34 (JL X JL)	2367/17 (AV)
H	1/39 (UR X UR)	2318/17 (AV)
I	33/17 (JL X JL)	2318/17 (AV)

TABLE 2
The Chemical Properties of Serdang Series Soils

Parameters	Value
$\text{pH}_{(\text{water})}$	4.5
Total N (g kg^{-1})	1.3
Bray-2 P (mg kg^{-1})	5.4
Organic C (g kg^{-1})	8.5
Cation Exchange Capacity (cmol(+) kg^{-1})	4.3
Exchangeable K (cmol(+)kg^{-1})	0.1
Exchangeable Ca (cmol(+)kg^{-1})	0.8
Exchangeable Mg (cmol(+)kg^{-1})	0.2

RESULTS AND DISCUSSION

Growth Performance

Most of oil palm characters, such as bunch yield, bunch weight and bunch number, which are economically important are controlled by polygene, and they are more susceptible to the influences of environment factors (Ooi *et al.*, 1973; Thomas *et al.*, 1969). Rafii *et al.* (2002) reported that there is a significant influence by environment on genetic variances in D × P progenies, as yield and bunch quality characters of the progenies would vary from one location to another. Hence, this experiment was conducted in such a way that oil palm seedlings were grown in a controlled environment with uniform fertilizer applications to detect the effect contributed by oil palm genotypes on the nitrogen uptake ability. These oil palm seedlings (Table 3) were produced in the same progeny by crossing or selfed different source of Dura, namely from Ulu Remis (UR), Johore Labis (JL), and Banting (BD), in order to produce mother palms, and these were crossed with Dura mother palm and the siblings of Avros Pisifera (AV) originated from BM 119 (1316) Avros Pisifera. The dry matter ($p = 0.69$) and the total nitrogen content ($p = 0.54$) of oil palm seedlings at 6 months old were not significantly affected by genotypes, and this indicated that the six-month old oil palm seedlings do not possess enough difference in their growth performance to significantly highlight the difference between each genotype. However, when the same experiment was repeated with nine-month old seedlings, oil palm genotypes provoked significant effects to the plant total dry matter ($p < 0.01$) as well as the total accumulated N in plants ($p < 0.01$). As indicated by the total dry matter of oil palm seedlings, genotype A which built up dry weight of 115.82g showed significant superior growth performance compared the most of the genotypes which ranged from 84.25g to 106.34g, except for genotype F (127.78g), while all the other genotypes were not significantly different. Similarly, genotype A also showed a significantly higher total accumulated N content within the plant (2.86g) as compared to the other genotypes, ranging from 1.35g to 2.1 g per plant. Despite the fact that the total dry matter and the total accumulated N in the plant were not significant for the 6 months old oil palm seedlings, a similar trend was observed throughout the data as compared

TABLE 3
The Effect of Genotype on Total Dry Matter and Total Nitrogen of Oil Palms

Genotypes	Total Dry Matter (g plant^{-1})				Total N (g plant^{-1})			
	6 months		9 months		6 months		9 months	
A	71.83	A	155.82	a	1.47	a	2.86	A
B	54.29	A	101.48	b	1.41	a	1.80	B
C	68.66	A	84.25	b	1.46	a	1.54	B
D	61.67	A	84.50	b	1.35	a	1.73	B
E	69.43	A	104.13	b	1.65	a	2.01	B
F	68.97	A	127.78	ab	1.53	a	2.10	B
G	58.38	A	99.76	b	1.18	a	1.35	B
H	67.01	A	106.34	b	1.49	a	1.92	B
I	58.64	A	105.90	b	1.34	a	1.90	B

Note: Values in a column with the same letter(s) are not significantly different according to the Student-Newman-Keuls Test at $P \geq 0.05$

to the 9 months old seedlings, in which genotype A achieved highest in dry matter, followed by genotype F, whereas genotypes A, E, and F recorded the highest total N content in both the 6 and 9 months old oil palm seedlings. These findings suggested that 9 month old oil palm seedlings could be a better indicator for the difference between oil palm genotypic characteristics.

The Effects of P Fertilizer on Plant's Nitrogen Uptake

According to Zin *et al.* (2007), oil palm's response to N fertilizer was severely restricted in the absent of P fertilizer. In this experiment, the interaction between oil palm genotype and P fertilizer was not observed ($P \geq 0.7$) for all the parameters measured. Hence, the discussion will focus on the main effect of P fertilizer. Total dry matter of the oil palm under the two different P levels recorded a similar trend, as shown by the oil palm growth performance (Table 4). This also indicates that P fertilizer does not contribute to significant difference in the growth of seedlings across all the genotypes for the 6-month old oil palm seedlings ($p = 0.51$), but significant effects were seen in 9-month old oil palm seedlings ($p = 0.02$).

It is important to note that the soil used as a planting medium in this experiment contained small amount of phosphorus, and the oil palm seedlings were planted in poly bag containing 30kg of soil. Consequently, due to the less P requirement of young oil palm seedlings, the phosphorus within the soil would minimize the effects of P fertilizer on the growth performance of young seedlings over a short period of time. The results of this study is concurrent with the findings of other researchers who have reported that N and P fertilizers contribute to increase in the dry matter productions in plants (Wilkinson *et al.*, 1999) and P has synergetic effects on nitrogen in term of yield (Zin *et al.*, 2007). However, at both ages of oil palm seedlings, P fertilizer does not contribute to any significant difference for the total N accumulation in the plant ($p = 0.58$ and 0.09). As mentioned earlier, the planting medium used in this experiment contained small amounts of phosphorus, and the seedlings planted in this experiment might not confront with P stress condition, and hence, the P fertilizer's effect would be suppressed as long as P threshold had not been overcome (Fong & Lee, 1998).

TABLE 4
The Effect of P Fertilizer to Total Dry Matter and Total Nitrogen in Oil Palms

	Total Dry Matter (g plant^{-1})				Total N (g Plant^{-1})			
	6 months		9 months		6 months		9 months	
With P	65.98	a	117.02	a	1.46	a	2.01	a
Without P	62.67	a	96.88	b	1.40	a	1.76	a

Note: Values in a column with the same letter(s) are not significantly different according to the Student-Newman-Keuls Test at $P \geq 0.05$.

Nitrogen Derived from Fertilizer (NdFF)

Since oil palm is a major source of oil and fat for human, breeders have been working to shorten the process of breeding selection cycle, and also accelerate the breeding progress (Soh, 2004). However, being a perennial crop, oil palm breeding cycle could easily require 10 to 15 years (Norziha *et al.*, 2008). Additionally, only 148 palms can be planted on 1 hectare, and thus, oil palm progeny trails require much larger areas, cost, and maintenance. This study incorporates the isotope labelling technique in order to provide more genotypic information for plant breeders. In the previous studies, ^{15}N isotopes had been applied as tracers in estimating N_2 fixation (Alves *et al.*, 2000; Chalk, 1996; Hamilton *et al.*, 1991; Danso, 1986; Chalk, 1985), N deposition in field (Böhme & Russow, 2005) and to determine the leaching of nitrogen from fertilizers (Bergstrom & Kirchmann, 2004). Additionally, ^{15}N isotopes were also used in quantifying nitrogen uptake efficiencies of Arabidopsis (Chardon *et al.*, 2010), and nitrogen recovery efficiencies of coated urea in potato fields (Zvomuya *et al.*, 2003).

As shown in Table 5, the results revealed that only the N yield derived from labelled fertilizer (0.31g) of 9-month old oil palm seedlings is significantly higher ($p < 0.01$) as compared to the other genotypes, while the 6-month old oil palm seedlings are not significantly different ($p = 0.90$) across the genotypes. The other parameters, namely total N increase and total %Ndff at 6-month old ($p = 0.72$ and 0.73, respectively) and 9-month old ($p = 0.56$ and 0.22, respectively) seedlings, were also not significantly different between the genotypes. Generally, genotype A demonstrated superior N uptake performance, which could be observed from both the 6-months old and 9-months old oil palm seedlings.

Meanwhile, the ^{15}N labelling technique has provided an insight into the nitrogen use efficiencies (NUE) of the oil palm, whereby genotype A achieved the highest NUE as much as 6.12%, genotype F at 3.27%, while the other genotypes utilized less than 2% of the applied labelled fertilizer. Genotype A contained as much as 10.48% of ^{15}N within the plants as compared to the total N of the plant, followed by genotype F which contained 7.62% of ^{15}N in total N of the plants. The specific Dura from Johore Labis was selected due to its favourable characteristics, such as very big bunch size with low bunch number, thinner shell, and Ulu Remis Dura was chosen because of its ability to reduce sex ratio without reducing the yield. Meanwhile, Banting Dura was used in crossing due to the higher oil content in its fruit bunches, thick mesocarp, and thin shell. Avros Pisifera 2367/17 and 2318/17 were close siblings whose parents were selected due their favourable characteristics, such as high general combining ability and exceptional vigorous vegetative growth.

Genotype A, which was produced from UR X JL X AV, could have higher genetic diversity to inherit more favourable genotypic characteristics as compared to the genotypes which were produced through selfed mother Dura crossed with Avros

TABLE 5
The Effects of Oil Palm Genotype on Total N Increase, Total %Ndff, and N Yield Derived from ^{15}N Fertilizer

Genotypes	Total N Increase (g plant^{-1})				Total %NdFF				N Yield Derived from ^{15}N Fertilizer (g plant^{-1})			
	6 months		9 months		6 months		9 months		6 months		9 months	
A	0.50	a	1.15	a	47.11	a	27.49	a	0.17	a	0.30	a
B	0.37	a	0.73	a	44.57	a	32.43	a	0.15	a	0.09	b
C	0.53	a	0.36	a	48.23	a	32.84	a	0.15	a	0.07	b
D	0.45	a	0.71	a	51.81	a	35.91	a	0.16	a	0.09	b
E	0.42	a	0.69	a	49.25	a	30.43	a	0.16	a	0.09	b
F	0.51	a	0.67	a	45.87	a	27.69	a	0.16	a	0.16	b
G	0.36	a	0.43	a	45.48	a	29.43	a	0.12	a	0.03	b
H	0.54	a	0.59	a	44.45	a	27.79	a	0.17	a	0.09	b
I	0.29	a	0.78	a	44.59	a	28.14	a	0.11	a	0.07	b

Note: Values in a column with the same letter(s) are not significantly different according to the Student-Newman-Keuls Test at $P \geq 0.05$

Pisifera. Genotype E, which was also produced via mother palm produced from Banting Dura and Ulu Remis Dura crossed with AV, produced a similar favourable growth performance compared to the other genotypes, despite the fact that data were not statistically higher. These two genotypes generally showed higher growth performance average as compared to the other genotypes, despite the fact that genotype E was not statically different from the other genotypes. The result could be partially influenced by the short growing periods of the oil palm which had prohibited the oil palm seedlings to express more differences in terms of their growth performance caused by genetic superiority. Meanwhile, the differences between genotypes A and E with the others could be more prominently distinguished if the experiment time period had been extended. On the other hand, genotype F also showed a promising growth performance and a high nitrogen utilization rate as compared to genotype A. As much as 25000 palms were visually selected based on the well-formed bunches and appearances, and Dura was selfed and sib-mated to produce the mother palm for genotype F. Hence, it is favourable to observe the superior growth of this particular genotype as inherits of vigorous parental characteristic. These genotypes of oil palm were newly developed by Sime Darby Research Sendirian Berhad, and hence, the data obtained from this experiment could be used as a reference for plant breeders so as to produce oil palm seedlings with higher nitrogen uptake ability. Nonetheless, due to the variations in the nitrogen uptake of oil palms, more study are still required to fully understand oil palm physiologies which correspond to genotypic characters, and consecutively identify oil palm genotypes with high N uptake.

CONCLUSION

It is important to note that there is no interaction of P fertilizer with oil palm genotypes. Hence, the P fertilizer gives a uniform effect to all the genotype growth performances, and P fertilizer has synergistic effects on the efficiency of oil palms' nitrogen uptake. Oil palm seedlings at 6 months old did not give a clear indication of the difference in the growth performance across all oil palm genotypes. Meanwhile, the dry matter, total N, and even N derived from the ^{15}N labelled fertilizer were not significantly different across the genotypes; hence, it is safe to conclude that 6-month old oil palms are not sufficient enough to reveal the difference in their genotypic characters. However, 9-months old oil palm seedlings showed significant difference in their total dry matter, total N and N yield derived from the labelled fertilizer across the different genotypes. In particular, Genotype A recorded the highest total dry matter (155.82 g/ plant), highest total accumulated N in plants (2.86 g) and N yield derived from the ^{15}N labelled fertilizer in plants (0.30g), highest nitrogen use efficiencies (6.12%), and highest ^{15}N concentration within the plants (10.48%). Meanwhile, the ^{15}N isotope labelling technique could provide a better insight into the oil palm seedlings' nitrogen uptake efficiencies. In order to detect the differences between oil palms genotypic variability, it is advisable to utilize slightly matured oil palm seedlings, in which the stress symptoms could be more profound than that of the young seedlings. It is also wise to extend the period of palm oil growth in order for the oil palms to show greater difference in their genotype superiority.

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