

## Evaluating the Variability of Gafsa Phosphate Rock Uptake by Oil Palm Genotypes at Nursery Stage

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### ABSTRACT

The application of phosphate fertilizer in crops accounts for 90% of the total world's mineral phosphate mined and the reserve of phosphate rock in the world continues to deplete. Thus, it is imperative to understand the potential of different oil palm genotypes in taking up added phosphate fertilizers in order to conserve this particular non-renewable resource. In this study, the P-32 reverse isotope dilution method was used in a greenhouse to assess nine different oil palm genotypes in taking up phosphate fertilizer (Gafsa Phosphate Rock) for a period of 6 months. The measurements of the dry weight and P accumulation in plant were conducted during the course of the study. However, the two measurements did not clearly show the difference in the phosphate uptake after the application of Gafsa Phosphate Rock. In more specific, the accumulation of phosphate in the different genotypes only demonstrated significant difference between the genotypes (25/49 x 2367/17 and 9/103 x 2318/17) in the first three months. However, the P-32 reverse dilution method revealed a significant difference in the phosphate fertilizer uptake for the genotypes at 0-3 and 3-6 months. Over the six months' period, the genotypes were observed to take up around 14 to 46 percent of the phosphate added fertilizer. The potential of the different oil palm genotypes to take up phosphate from fertilizer, according to the P-32 reverse isotope dilution method, could be ranked as 2/35 x 2367/17  $\geq$  19/19 x 2367/17  $\geq$  2/209 x 2367/17 > 25/49 x 2367/17  $\geq$  9/103 x 2318/17  $\geq$  33/17 x 2318/17 > 14/34 x 2367/17  $\geq$  23/34 x 2367/17  $\geq$  1/39 x 2318/17. Therefore, the P-32 reverse isotope dilution method could serve as a useful means of assessing the phosphate uptake potential of oil palm seedlings. This study implies that oil palm seedlings, with better phosphate uptake efficiency from fertilizer, can help to reduce fertilizer wastage and contamination of water source, and obtain a better ability to cope with phosphate deficiency.

**Keywords:** Oil palm, Gafsa Phosphate Rock, P uptake, P-32 reverse isotope dilution

### INTRODUCTION

Phosphorus is one of the most essential elements for growth and functioning of plants. Phosphorus deficiency is considered to be one of the major limitations in the crop production on a global scale, especially in the tropical acidic soil (George *et al.*, 2006; Raghothama and Karthikeyan, 2005). Malaysian soils (namely, Ultisols and Oxisols), like most

other tropical soils, are known to be highly weathered, acidic, and inherently low in P and have high P fixing capacities. Ferric oxide and aluminium hydroxide are the primary sorbents for phosphate in soil and these could lead to substantial P-fixation (Zaharah and Sharifuddin, 1979; Goh and Chiew, 1995; Sallade and Sims, 1997; Wilson *et al.*, 2004). Although oil palm requires a smaller amount of P than nitrogen and potassium, this element has been reported

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to have a synergistic effect with other nutrients on oil palm yield (Foster *et al.*, 1988; Foster and Prabowo, 1996). High yield and increase in the production of oil palm in Malaysia are largely dependent upon the application of fertilizers because oil palm requires high demand, uptake, and removal of nutrient (Von Uexkull and Fairhurst, 1991; Goh and Hårdter, 2003). A direct application of phosphate rock in oil palm plantation has been a standard practice since the 1930s (Zaharah *et al.*, 1997). In particular, phosphate rock is preferred in acidic soil as it is nearly as effective as water-soluble P fertilizer and more cost effective (Chien and Menon, 1995). Runge Metzger (1995) claimed that the consumption of world high-grade phosphate rocks might cause depletion within 60 to 90 years. Therefore, to improve and conserve the phosphate deposit left in this world, it is important to understand the ability of different oil palm genotypes in taking up phosphate in soil. Even at the nursery stage, the requirement for fertilizer by oil palm seedlings with 2 to 5 leaves is around 30 g of NPK 12-12-17 fertilizers for every 4 weeks (Gillbanks, 2003). Many studies have reported that the efficiencies of plants to extract and uptake P markedly vary between the cultivars of various crops (Narang *et al.*, 2000; Gahoonia and Nielsen, 1996; Horst *et al.*, 1996; Manske *et al.*, 2000; Osborne and Rengel, 2002). Thus, this experiment was carried out to evaluate the potential of various oil palm genotypes in taking up phosphate fertilizer using the P-32 reverse dilution method.

## MATERIALS AND METHODS

The genotypes of oil palm seedlings obtained in this study were the new varieties developed by Sime Darby Research Sendirian Berhad (Table 1). The seedlings used were germinated from seeds and maintained according to generic fertilizer programme for oil palm seedlings (Gillbanks, 2003) before subjected to treatment application. Five-months-old oil palm seedlings from nine genotypes were arranged in Randomized Complete Block Design (RCBD) inside a greenhouse, with and

without phosphate fertilizer treatments in four replicates for each genotype. The fertilizer used was Gafsa Phosphate Rock (GPR) (Table 1). A total of 144 bags containing 30 kilograms of soil (Serdang Series) were prepared. The fertilization programme to cover three month's period of the study consists of 4.9 g nitrogen, 5.0 g potassium, and 3.9 g magnesium applied as urea, Muriate of Potash and Kieserite, respectively and micronutrients from AJIB® at 20 g. Only half of 144 bags were applied with 6.7 g phosphorus as Gafsa Phosphate Rock (13.2% P), while the remaining were without any addition of P to be used as control for each oil palm genotypes and to reflect the P derived from the soil. The seedlings were left to grow for a period of three months so as to allow for the uptake of fertilizer. Using the same procedure, another set was prepared with 5-months-old seedlings to be harvested at 6 months after planting. Hence, the duration for the observation of the study was 6 months, comprising two harvests. For the set to be harvested at 3 months, 72 bags of soil were labelled as 100  $\mu$ Ci of P-32 with 2 mg of  $\text{KH}_2\text{PO}_4$  added as a carrier. Meanwhile, for the set to be harvested at 6 months, 150  $\mu$ Ci of P-32 with 1 mg of  $\text{KH}_2\text{PO}_4$  as carrier was added to each oil palm seedling for the remaining 72 plants after the first set had been harvested. It is important to note that destructive sampling was conducted at three and six months after planting. Only the shoot of the seedlings were harvested. The samples of the plant tissues were separated into rachis and leaves, prior to drying at 70°C until constant weights were achieved. The dry weight of each sample was recorded using balance. Five grams of finely cut samples from rachis and leaves were ashed at 350°C for two hours and subsequently at 500°C for three hours. The ash was dissolved in 20 ml 2N HCl and filtered (Advantec 5C, Toyo Roshi Kaisha, Ltd., Japan). The P-32 activity was measured using Cerenkov counting with Liquid Scintillation Counter (LSC) (model Tri-Carb 3100TR by Packard-Packard BioScience Co). The inorganic P in the plant tissue was determined using the method proposed by Scheel (1936).

TABLE 1  
9 new varieties developed by  
Sime Darby Research Sdn. Bhd.

No.	Genotypes
1	14/34 x 2367/17
2	2/35 x 2367/17
3	2/209 x 2367/17
4	19/19 x 2367/17
5	25/49 x 2367/17
6	9/103 x 2318/17
7	23/34 x 2367/17
8	1/39 x 2318/17
9	33/17 x 2318/17

The specific activity was calculated by the disintegrations per minute (DPM) of the sample divided by the total inorganic P in the sample (IAEA, 2001). Phosphorus derived from the fertilizers (PdfF) was calculated using the isotope dilution formula described by Zapata and Axmann (1995), whereby:

$$\text{PdfF (\%)} = 100 \left[ \frac{\text{specific activity of plants with fertilizers}}{\text{specific activity of plants without fertilizer}} - 1 \right]$$

The percentage of phosphate derived from fertilizer (PdfF) was subjected to arc sine transformation. The P accumulation in plant was calculated as the product of rachis P and leaves P concentration times the respective dry biomass of the rachis and leaves. A two-way analysis of variance (ANOVA) was used to analyze the experimental data. For separation of means, TUKEY's HSD (honestly significantly different) test was carried out. All the analysis was done using the SAS package version 9.0 for Windows.

## RESULTS AND DISCUSSION

### *Dry Matter Yield and P Accumulation*

The soil has a  $\text{pH}_{(\text{water})}$  of 4.5, Bray-2 extractable P of 5.4 mg/kg, organic carbon of 0.85%, exchangeable K 0.1 cmol(+)/kg, exchangeable Ca 0.8 cmol(+)/kg, exchangeable Mg 0.2 cmol(+)/kg, and Cation Exchange Capacity of 4.3 cmol(+)/kg. The soil texture analysis showed 38% clay, 10% silt, and 52% which gave a textural class of sandy clay. The application of Gafsa Phosphate Rock to nine genotypes gave a similar weight accumulation over six months (Table 3) which yielded the total weight between 114.1 g to 139.4 g. There was no significant difference ( $p < 0.05$ ) in term of weight at the intervals of 0-3 months and 3-6 months, and this suggested that there was no difference in the response of growth for all the genotypes assessed. However, the growth response of the genotypes at 3 months showed a significant difference ( $p < 0.05$ ) in the accumulation of P (Table 4) between the genotypes (25/49 x 2367/17 and 19/19 x 2367/17) which accumulated 177.2 mg P per plant and 116.2 mg P per plant, respectively. Genotype 19/19 x 2367/17 gave the lowest P accumulation in dry weight. At 6 months (Table 4), the differences in the P accumulation between the genotypes were not statistically detected. At six months, the response of P accumulation in the genotypes was similar to that at 3 months, with 25/49 x 2367/17 (highest mean) being significantly different ( $p < 0.05$ ) and 19/19 x 2367/17 (lowest mean) as the least. According to Fong and Lee (1998), the level of P in plants may not be a good indication of crop performance due to the fact that the P applied will no longer increase the amount of P in plant once the threshold of P level is attained. Overall, there was an increase in the dry weight for all the

TABLE 2  
Details of Gafsa Rock Phosphate

P source	Total P (%)	Total Ca (%)	Solubility as percent of rock	
			2% Formic acid	2% Citric acid
Gafsa Phosphate Rock	13.4 ± 0.3	31.8 ± 0.6	20.8 ± 0.2	11.7 ± 0.1

TABLE 3  
Dry matter weight of oil palm seedlings accumulated at different times of harvest

Genotype	g per plant					
	0-3 months		3-6 months		Total (1-6 months)	
14/34 x 2367/17	53.53	A	85.83	A	139.36	A
2/35 x 2367/17	52.06	A	70.65	A	114.13	A
2/209 x 2367/17	51.75	A	72.67	A	119.45	A
19/19 x 2367/17	44.34	A	80.94	A	120.99	A
25/49 x 2367/17	56.68	A	73.26	A	129.94	A
9/103 x 2318/17	58.32	A	80.65	A	138.97	A
23/34 x 2367/17	55.68	A	75.05	A	130.73	A
1/39 x 2318/17	47.38	A	71.68	A	115.82	A
33/17 x 2318/17	52.37	A	71.83	A	122.8	A

\* Mean with the same letter is not significantly different at 5% level by TUKEY

TABLE 4  
Total P accumulated by oil palm seedlings at different times of harvest

Genotype	mgP per plant					
	0-3 months		3-6 months		Total (1-6 months)	
14/34 x 2367/17	162.69	A B	171.19	A	333.89	A
2/35 x 2367/17	140.80	A B	192.94	A	333.74	A
2/209 x 2367/17	148.62	A B	200.40	A	349.03	A
19/19 x 2367/17	116.15	B	166.65	A	282.80	B
25/49 x 2367/17	177.22	A	211.37	A	388.60	A
9/103 x 2318/17	164.30	A B	172.20	A	336.50	A
23/34 x 2367/17	155.57	A B	216.89	A	372.45	A
1/39 x 2318/17	147.40	A B	205.27	A	352.67	A
33/17 x 2318/17	154.20	A B	161.49	A	315.70	A

\* Mean with the same letter is not significantly different at 5% level by TUKEY

genotypes, i.e. approximately 20 percent from 3 to 6 months. The P accumulated dry weight also demonstrated an increase of approximately 30 percent for the genotypes 2/35 x 2367/17, 2/209 x 2367/17, 23/34 x 2367/17, 1/39 x 2318/17, and approximately 5 percent increase for the genotype 14/34 x 2367/17, 9/103 x 2318/17, and 33/17 x 2318/17.

### PHOSPHATE DERIVED FROM FERTILIZER (PDFF)

According to Zapata and Axmann (1995), isotopic technique is an effective method to study crop genotypic differences in phosphate uptake from phosphate efficiency. Isotopic data such as PdfF is a yield-independent parameter that provides an accurate measurement of the available P to a crop from the applied phosphate source (Broeshart, 1974; Kucey and Bole, 1984). Gahoonia and Nielsen (1998) reported that the approach involving the use of the P-32 reverse dilution method provides information on nutrient uptake of plants is evident and reliable. Among other, the study by Zaharah *et al.* (1997) revealed that the P-32 isotope technique provided a good understanding of evaluating the efficacy of various phosphate fertilizer sources for oil palm seedlings. Other similar studies using P-32 were also done to assess phosphorus efficiency in wheat (Hayes *et al.*, 2004). There was a significant difference ( $p < 0.05$ ) in the phosphate uptake from fertilizer for the genotypes assessed (Table 5). The accumulations of P in the dry weight of genotypes only demonstrated a significant difference ( $p < 0.05$ ) between the genotypes 25/49 x 2367/17 and 9/103 x 2318/17 in the first three months. The technique of using P-32 reverse isotope dilution method (Zapata and Axmann, 1995) to examine the phosphate derived from fertilizer of various oil palm genotypes showed significant difference ( $p < 0.05$ ) at 3 and 6 months (Table 5). Over the six months, 14% to 46% of the P uptake from fertilizer were observed. The highest phosphate uptake at 3 month was by genotype 2/209 x 2367/17, which was 46.82% or 66.75 mg P per plant. At 6 months, the highest uptake

was shown by the genotype 2/35 x 2367/17. Six months of observation revealed that the percentages of the phosphate uptake by the oil palm genotypes could be ranked as in Table 5 (i.e.  $2/35 \times 2367/17 \geq 19/19 \times 2367/17 \geq 2/209 \times 2367/17 > 25/49 \times 2367/17 \geq 9/103 \times 2318/17 \geq 33/17 \times 2318/17 > 14/34 \times 2367/17 \geq 23/34 \times 2367/17 \geq 1/39 \times 2318/17$ ), which ranged from 12% to 46%. A relative comparison in the amounts of phosphate derived from fertilizer (mg P per plant) among genotypes over 6 months was ranked similar to the P uptake percentage. 2/35 x 2367/17 recorded 153mg P per plant as the highest P in the plant or 2.3 percent of the P added at the beginning of experiment, while 1/39 x 2318/17 recorded 74.05 mg P per plant or 1.1 percent of the added P.

### Implication of the Study

Phosphorus availability is defined as the phosphate in soil solution that is available by desorption and dissolution processes for the uptake by plants in the terrestrial and aquatic ecosystem (Sharpley, 2000). However, the fate of Phosphate in soil is normally precipitated and unavailable for plant use (Marschner, 1995). In acidic condition, aluminium and iron are the most important elements responsible for the immobilization of P. These properties could result in substantial P-fixation with dissolved Fe, Al, and Mn ions to form insoluble hydroxy phosphate precipitates (Fontes *et al.*, 1996; Brady *et al.*, 2004). In addressing this problem, the availability of phosphorous can be maintained or built up by adding phosphate fertilizers such as phosphate fertilizer. Interestingly, the application of Gafsa Phosphate Rock is subjected to the same fate of P-fixation in soil, yet this study has shown different genotypes are able to uptake phosphate fertilizer at a variable rate, and 10% to 40% of phosphate from fertilizer was also observed. This finding suggests that fertilizer is more available for uptake in some plants and thus possesses higher phosphate uptake efficiency. Thus, this could be interpreted as part of the measure to reduce fertilizer wastage at the nursery stage.

TABLE 5  
P derived from fertilizers (percentage and mg/plant) in oil palm seedlings at different times of harvest

Genotypes	PdfF (%)				PdfF (mg/plant)			
	0-3 months	0-6 months	Average 1-6 months	0-3 months	0-6 months	0-6 months	Total 1-6 months	
14/34 x 2367/17	22.57	8.96	15.66	36.36	15.34	52.29	DE	DE
2/35 x 2367/17	44.48	48.92	45.96	60.53	94.39	153.39	A	A
2/209 x 2367/17	46.82	23.59	34.25	66.75	47.27	119.54	A	B
19/19 x 2367/17	45.4	48.07	45.93	50.86	80.09	129.88	A B C	A B
25/49 x 2367/17	29.06	31.66	30.08	50.51	66.92	116.89	A B C	B
9/103 x 2318/17	28.42	31.90	29.96	46.05	54.93	100.83	B C D	B C
23/34 x 2367/17	20.42	7.22	13.73	31.49	15.66	51.137	E D F	E D
1/39 x 2318/17	14.12	10.61	12.32	20.68	21.78	43.46	E F	E D
33/17 x 2318/17	8.51	38.46	23.46	13.04	62.09	74.05	F	B C

\* Mean with the same letter is not significantly different at 5% level by TUKEY  
PdfF: Phosphate derived from fertilizer

Phosphate moves in liquid phase through soil via mass flow and diffusion before it is taken up by roots (Tinker, 1976; Barber, 1995). According to Gillbanks (2003), a normal practice at the nursery stage requires 8 mm to 10 mm of irrigated water to seedlings each day. The movement of water in a seedling polybag often experiences infiltration, percolation, and runoff. Thus, phosphorus fertilizer applied closed to the surface is susceptible to loss by surface runoff (Goh and Hardter, 2003). If a considerable amount of phosphate accumulates in soil solution, there will be eutrophication to other water source (Johnston and Dawson, 2005) and this leads to water problem in/near nursery area. Therefore, seedlings with better phosphate uptake from fertilizer might help to mitigate this problem.

In addition, selecting seedlings with better phosphate uptake may help to rectify phosphate deficiency at a much faster rate to ensure a proper growth of the seedlings. In dealing with soils having low availability of phosphate, the current management practice of oil palm and other crops includes the application of phosphate fertilizer (Zaharah *et al.*, 1997). Phosphate acquisition and translocation greatly influence the quality and yield of crops. Moreover, insufficient P application during the establishment may lead to poor palm development such as stunting (Goh and Hardter, 2003).

The different uptake potentials in phosphate by various genotypes can be explained in several perspectives. In general, it is believed that P-efficient cultivars are able to acquire more phosphate via the induction of phosphate Pi transporters with a higher affinity for phosphate uptake in roots allowing the plant to absorb more P from lower concentrations in the soil solutions (Mimura, 1999; Smith *et al.*, 1997). Moreover, the exudation of compounds into rhizosphere can increase the mobility of P in soil, and this includes the increase of extracellular acid phosphatase activity of roots and other organic acid including carboxylates (Bhadraray *et al.*, 2002; McLachaln, 1980; Rovira, 1969; Hayes *et al.*, 2004). Meanwhile, the changes in the root morphology and architecture such as the

root system enlargement, increased root-length system, and arbuscular mycorrhiza establishment to permit the plant to explore a greater volume of soil can also increase phosphate uptake efficiency (Romer *et al.*, 1988; Williamson *et al.*, 2001; Yan *et al.*, 2001). Therefore, more studies on the physiologies of oil palm should be carried out to understand the reasons for better phosphate uptake in some genotypes, as it has been demonstrated in this study.

## CONCLUSIONS

The dry weights and P accumulation of different genotypes did not give a clear estimation on the phosphate uptake by the oil palm seedlings. The dry weight of different genotypes was rather similar while the P accumulation in plant showed only a significant difference between two genotypes over six months (namely, genotype 25/49 x 2367/17 and genotype 19/19 x 2367/17). Apparently, these measurements are not good enough to differentiate the phosphate uptake from fertilizer or soil and ultimately cannot be used to determine the potential of oil palm genotypes to take up phosphate from fertilizer. Meanwhile, the P-32 reverse dilution method provides a way to differentiate the phosphate derived from fertilizer or soil. The potential of different oil palm genotypes to take up phosphate from fertilizer, according to the P-32 reverse isotope dilution method, can be ranked as  $2/35 \times 2367/17 \geq 19/19 \times 2367/17 \geq 2/209 \times 2367/17 > 25/49 \times 2367/17 \geq 9/103 \times 2318/17 \geq 33/17 \times 2318/17 > 14/34 \times 2367/17 \geq 23/34 \times 2367/17 \geq 1/39 \times 2318/17$ , ranging from 46 percent to 12 percent. Such ranking is also similar for the mg of phosphate uptake per plant. Therefore, it is evident that the potential of phosphate uptake by various palm oil genotypes cannot be measured using conventional dry weight and P accumulation in plant, but the radioisotope technique provides a better insight into the potential of various oil palm genotype seedlings to uptake phosphate from fertilizer. This study implies that oil palm seedlings with better phosphate uptake efficiency from fertilizer can help to reduce fertilizer wastage and

contamination of water source, in addition to a better ability to overcome phosphate deficiency. Nonetheless, more studies are required to fully understand the physiologies of oil palm so as to reveal a better phosphate uptake of some genotypes.

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