# Optimizing calcium levels for oil palm seedlings in solution culture

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Abstract Oil palm seedlings grown in solution culture exhibited the best growth and physiological responses when treated with 1000 ppm calcium (Ca) from calcium chloride (CaCl<sub>2</sub>). This concentration is significantly enhanced total biomass accumulation and improved vegetative parameters including seedling height, girth, root length, and number of leaves, compared to lower and higher Ca treatments. At 12 weeks, seedlings at 1000 ppm Ca showed significantly greater root length (27.08 cm), seedling height (41.85 cm), and girth (15.89 mm), alongside increased chlorophyll content (SPAD value of 55.48), indicating improved photosynthetic efficiency. Excess Ca (more than  $\geq 2500$  ppm) inhibited nutrient uptake, particularly nitrogen (N), phosphorus (P), and magnesium (Mg), due to antagonistic interactions, whereas 1000 ppm Ca maintained optimal macro-nutrient balance in both shoot and root sections. Ca concentration beyond this level led to reduced nutrient availability and poor growth performance, with 7000 ppm Ca showing the least favorable outcomes. The uptake of Ca was positively correlated with increased Mg concentration at optimal levels but decreased under excessive Ca conditions. The study demonstrated that 1000 ppm Ca not only optimizes growth parameters and biomass accumulation but also supports effective nutrient partitioning, making it the ideal concentration for oil palm seedling cultivation in hydroponic systems. These results provide critical insight for improving nutrient management strategies in oil palm nurseries to enhance early growth performance.

Keywords: Calcium, Oil palms, Solution culture, Optimum level

# Introduction

The oil palm (*Elaeis Guineensis* Jacq.) is the most important crop, accounting for 39% of global vegetable oil production (USDA, 2014). Malaysia's

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plantations, covering around 5.23 million hectares, cover 30% of the global palm oil supply (MPOB, 2013). Other important producers are Thailand, Colombia, Nigeria, Papua New Guinea, and Ecuador. Providing optimal mineral nutrition can increase crop output and quality. Mineral nutrition ensures crop plant growth and productivity by supplying, absorbing, and utilizing vital nutrients (Fageria *et al.*, 2010). Oil palm requires a balanced and appropriate supply of macro- and micronutrients for optimal growth and output. Efficient fertilizer use is crucial for achieving optimal economic and sustainable oil palm yields.

Calcium (Ca) is a secondary plant nutrient but is just as essential for plant growth, even though those elements are required in negligible amounts compared to macronutrients. While Ca is part of every plant cell, it keeps cell walls upright and increases fruit set and quality. Marschner (2012) states that calcium is a big divalent cation with a hydration energy of 1577 J/mol<sup>-1</sup> and an ionic radius of 0.412 nanometers when it is hydrated. In the natural world, calcium shortage is quite uncommon; nonetheless, high calcium restricts plant communities to calcerous soil. The Ca is taken from the roots and delivered through the xylem to the shoot from the soil solution. It can go through the root via either apoplastic means of passing through spaces between cells or symplast means of travelling through the cytoplasm of cells that are connected by plasmodesmata (Keller, 2020). According to White and Broadley (2003), a component of the Ca is tightly attached to the structures that are in the apoplasm, while another fraction is interchangeable with the cell walls and the surface of the plasma membrane. For structural activities in the cell wall and membranes, as well as for inorganic and organic anions in the vacuole, it is necessary (Marschner, 1995), and for intracellular messengers in the cytoplasm.  $Ca^{2+}$  is a divalent cation, which means that it is required for these activities.

As a secondary nutrient and a crucial component of plant nutrition, Ca has recently garnered a lot of attention in the fields of plant physiology and molecular biology. Ca can be presented in mature leaves and other high-concentration areas (over 10% dry weight) without causing any harm to the plant or significantly slowing its growth. Cell walls (apoplasm) contain the most total calcium in plant tissue, in contrast to other macronutrients. The unusual distribution is primarily due to the large number of Ca binding sites in the cell wall (Marschner, 2012). There is a relationship between the supply of Ca and the total Ca proportion in different binding types, such as water-soluble, pectate, phosphate, oxalate, and residue (Lopes and Guimarães Guilherme, 2016).

Numerous research has examined the balance of nutrient uptake in soilbased plants (Bloomfield *et al.*, 2014; García-Palacios *et al.*, 2014; Koorem *et al.*, 2014). However, in soil-based plant experiments, there is a complicated interplay between the plant, the soil, and microbes that may result in competing effects and do not accurately reflect the actual Ca nutrients taken up and utilized by the plants. Furthermore, the soil medium contains a complicated interaction of ions within soil particles (Conn *et al.*, 2013). However, Ca deficiency was a central problem in the tropics due to high rainfall, excessive Ca-leaching, and low soil pH. The main reason for Ca deficiency is typically a lack of this Ca element in the soil due to an increased concentration of competing ions that function as calcium ion antagonists, which are hydrogen (H<sup>+</sup>), potassium (K<sup>+</sup>), magnesium (Mg<sub>2</sub><sup>+</sup>), and ammonium (NH<sub>4</sub><sup>+</sup>) ions (El Habbasha and Ibrahim, 2015).

Hydroponically grown plants cultivated in a soil-free system with a controlled nutrient-rich solution have grown faster than soil-based plants. They are also an ideal medium for evaluating plant physiology (Conn *et al.*, 2013). Correct nutrient management at the nursery stage can prevent waste and reduce fertilizer losses. Hydroponics provide higher yields per unit area, year-round production, and easier harvesting due to minimal contamination from pollutants, pests, and pathogens (Pardossi *et al.*, 2006). Additionally, the nutrient profile of the growth medium is easily manageable. This study investigated the effects of various Ca concentrations on growth performance, biomass accumulation, and nutrient uptake in plant tissues of oil palm seedlings cultured in solution culture.

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#### Materials and methods

#### Study site

The study was carried out in a transgenic glasshouse of Universiti Putra Malaysia, located in Serdang, Selangor (2°59'33.1"N, 101°43'19.0" E)

# Plant materials and growth conditions

In this work, the seeds of oil palms, namely Elaeis guineensis Jacq. Also known as Deli *dura* × AVROS *pisifera* ( $D \times P$ ), they were obtained from the Malaysia Palm Oil Board (MPOB, 2013) Research Station located in Kluang, Johor. These seeds were purchased and harvested. The 'Munchong' series soils were filled with very fine, kaolinitic, hyperthermic, red, and yellow Tipik Tempalemoks, and all the seeds were sown and sprouted in black polybags (5 cm × 8 cm). The soil was collected from Ladang Taman Pertanian, Universiti Putra Malaysia, Puchong, Selangor.Approximately ninety days after the seedlings

were sown, the oil palm seedlings were transplanted into a container measuring 47 cm  $\times$  31 cm. This container was filled with an inert growth medium. It included Hoagland solutions with a pH of 6.0, with elemental concentrations of 210, 31, 234, 200, 48, 0.5, 5, 0.5, 0.01 and 0.05 ppm for N, P, K, Ca, Mg, B, Fe, Mn, Mo, and Cu, respectively, as described by Hoagland and Arnon (1950).

The polypropylene trays were used to cultivate the oil palm seedlings that had germinated. These trays were covered with a black plastic sheet on top of the container and both sides of the container. Wool and distilled water were used to maintain its moisture, and it was removed and changed every three days. The Transgenic Unit of the Institute of Plantation Studies (IKP), Universiti Putra Malaysia, was the location where the experiment was carried out. The glasshouse was designed to provide a controlled environment for agricultural purposes. It offered a sensor that could detect changes in temperature as well as changes in relative humidity within a glasshouse. Hence, the relative humidity was maintained at 60 to 80% and the temperature at 30 to 36<sup>o</sup>C.

# Treatments and experimental design

To generate a stock solution for the Ca treatment that would be utilized in the Hoagland solution, 10,000 ppm of Ca was extracted from calcium chloride (CaCl<sub>2</sub>). The appropriate quantities of calcium solution were taken from these stocks to construct four treatments with varying concentrations of calcium. Hence, the treatments comprised six treatments, which include 200 (positive control), 1000, 2500, 4000, 5500, and 7000 ppm of Ca from CaCl<sub>2</sub> which were replicated sixteen times for each treatment in a Complete Randomized design (CRD).

This research was conducted with the purpose of determining the optimal concentration of calcium in pure solutions of oil palm seedlings. As a result, no treatment is needed for the addition of Ca as a negative control (0 ppm). However, the optimum Ca concentration cannot be reached if we provide sufficient Ca in the Hoagland solution. Furthermore, the effect of excessive addition of Ca on the growth of oil palm seedlings and its antagonistic with other nutrients will be examined since no data is available for the oil palm. Treatment in this research was modified based on results from previous studies by Nur Sabrina *et al.* (2012), which showed that the mixture of 2 mg/L Cu and 4000 mg/L Ca is the best supplementation treatment for oil palm growth and development to reduce oil palm disease.

# Measurement of vegetative growth

To attain the most optimal rate of vegetative development, the dependent variables that were examined in this study were the total number of fronds, the seedling height (cm), the rachis length (cm), the girth (mm), the root length (cm), and the SPAD chlorophyll content at two-week intervals starting at one week after planting. Based on the total number of leaves was determined by counting those leaves on the plant. The height of the seedling was determined by measuring a distance from the top of the soil to the very tip of the leaves on the third frond. From the point where the first rachis was ripped to the end of the rachis of the third leave, the length of the rachis was measured. The girth diameter of the seedlings was measured at the bole of the stem, one cm above the ground, using an absolute digital calliper (Mitutoyo, Japan). The measurement of the root's length was taken from the tips of the longest root to the root nodes, which are about below the stem's bole. Using a SPAD-502Plus meter (Konica Minolta, Japan), the chlorophyll content was measured three times on leave number three using the method described by Hardon *et al.* (1969).

The seedlings were divided into two parts: shoot- and root parts. The shoot part contained bole, stem, and leaves, while the root part contained the whole seedling root system. Nevertheless, the fresh weight of the oil palm seedlings was determined during destructive sampling, and their dry weight after oven drying was used to calculate total biomass. To prevent root injury, each seedling was delicately uprooted. According to Shamala (2010), the seedlings were individually wrapped and then dried in an oven at 70°C to a constant weight.

#### Tissue analysis

To digest plant tissue, tissue samples were digested utilizing a wet acid digestion process. This protocol was defined by the presence of concentrated sulfuric acids (H<sub>2</sub>SO<sub>4</sub>) and fifty per cent hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). For determining the levels of nitrogen (N), phosphorus (P), and potassium (K) in tissue samples, an Auto Analyzer (AA) was utilized. On the other hand, atomic absorption spectroscopy (AAS) was used to ascertain the presence of additional elements (Jones *et al.*, 1991). Nutrients concentration in oil palm parts were determined according to Jones *et al.* (1991) formulae.

## Data analysis

The data collected were subjected to statistical analysis by means of the two-way analysis of variance (ANOVA) that was included in the SAS 9.2

program. To determine whether there was a relationship between the growth of oil palm seedlings and the interaction between the different Ca concentration rates and the period that was being studied (weeks), a two-way analysis of variance was carried out. In this study, the development of oil palm growth was the dependent variable, while the independent factors were the varied Ca concentration rates and the amount of time (weeks). At a significance level of 5%, the least significant difference (LSD) was utilized to perform the means separation statistical analysis.

#### Results

#### Effect of Ca on total biomass

There was a significant difference ( $p \le 0.05$ ) between treatment applications for total biomass. At twelve weeks, the total biomass of oil palm seedlings decreased when the Ca concentration increased in Hoagland solution (Figure 1). During the study period, the total biomass in treatment 1000 ppm of Ca was significantly larger than 2500 ppm of Ca > 200 ppm of Ca > 4000 ppm of Ca > 5500 ppm of Ca > 7000 ppm of Ca. There was a significant difference between treatment 1000 ppm of Ca with 200 ppm of Ca (control), 4000 ppm of Ca, 5000 ppm of Ca, and 7000 ppm of Ca. But no significant differences between treatment 1,000 ppm and 2500 ppm of Ca. However, a massive increase in total biomass was observed in treatment 1000 ppm of Ca. These findings indicated that treatment 1000 ppm of Ca significantly increased the total biomass of oil palm seedlings compared to the other treatments.



Figure 1. Total biomass, 12 weeks after treatment with various concentrations of Ca: Data are means  $\pm$  standard error by using the LSD (p  $\leq 0.05$ )

# Effect of Ca on plant growth parameters

In oil palm seedlings, the use of Ca had a pronounced impact on the total number of leaves throughout the study period. Figure 2 shows that oil palm seedlings grown with a nutrient solution containing 1000 ppm of Ca had an increase in leaves count, seedling height, girth, and root length. There was an interaction between weeks and treatment implementation at  $p \le 0.05$ . At twelve weeks during the study period, the total number of leaves in treatment of 1000 ppm of Ca was 0.3% higher than 7000 ppm and followed by 4.8% of 2500 ppm, 5.9% of 4000 ppm, 6.8% of 200 ppm, and 9.4% of 7500 ppm, respectively (Table 1). There were no significant differences between treatments 1000 and 7000 ppm of Ca. However, a massive increase in the total number of leaves was observed in the treatment of 1000 ppm of Ca. These results showed that treatment 1000 ppm Ca enhanced the total number of leaves in oil palm seedlings much greater than the control and other treatments.

Similar trends were observed in the seedling height of the oil palms, which increased in number after treatment with different concentration rates of Ca (Table 1). As the research ended, the height of the seedlings in treatment of 1000 ppm of Ca was 7.5%, significantly higher than 2500 ppm, followed by 8.3% of 4000 ppm, 9.2% of 7000 ppm, 10.1% of 5500 ppm and, 14.3% of 200 ppm, respectively. In this study, the results showed that treatment of 1000 ppm of Ca considerably enhanced the seedling height in oil palm seedlings, which was higher than the control and other treatments.

In the girth of the oil palm seedlings, there was no interaction between weeks and treatment implementation at  $p \ge 0.05$ . However, during the study period, the girth of oil palm seedlings had significantly increased in size when applied with different rates of Ca concentration at  $p \le 0.05$  (Table 1). At twelve weeks, the girth of oil palm seedlings in treatment 1000 of Ca was found to be 5.9% significantly more significant than 2,500 ppm, followed by 6.8% of 200 ppm, 10.2% of 7000 ppm, 11.4% of 5500 ppm and, 14.5% of 4000 ppm, respectively. The results showed that treatment 1,000 ppm Ca increased girth in oil palm seedlings much more than the control and other treatments.

A similar pattern was recorded in the root length of the oil palm, which increased in number after treated with different concentration rates of Ca (Table 1). As the seedlings of the oil palm grew older, the disparity between applied treatments became more pronounced. At twelve weeks of reserach period, the root length of oil palm seedlings in treatment 1,000 ppm of Ca was found to be 3.5% significantly longer than 5500 ppm, followed by 4.7% of 7000 ppm, 15.4% of 4000 ppm, 19.5% of 2,500 ppm and, 23.3% of 200 ppm, respectively. These results revealed that treatment 1000 ppm of Ca gave the most extended root length in oil palm seedlings much more than the control and other treatments.



**Figure 2.** Physical symptoms after twelve weeks of treatment with varying Ca concentrations. *Notes.* T1 - 200 ppm; T2 - 1,000 ppm; T3 - 2,500 ppm; T4 - 4,000 ppm; T5 - 5,500 ppm; T6 - 7,000 ppm

**Table 1.** Effect of Ca on plant growth parameters with varied concentration of Ca at 12-weeks post treatment

Total number	Height (cm)	Girth diameter (mm)	Root length (cm)
of leaves			- · ·
6.17b	35.85c	14.81abc	20.77c
6.62a	41.85a	15.89a	27.08a
6.30ab	38.70b	14.95ab	21.80c
6.23ab	38.39bc	13.59c	22.92bc
6.00b	37.64bc	14.08bc	26.14ab
6.60a	38.00bc	14.27bc	25.80ab
	Total number of leaves 6.17b 6.62a 6.30ab 6.23ab 6.00b 6.60a	Total number of leaves         Height (cm)           6.17b         35.85c           6.62a         41.85a           6.30ab         38.70b           6.23ab         38.39bc           6.00b         37.64bc           6.60a         38.00bc	Total number of leaves         Height (cm)         Girth diameter (mm)           6.17b         35.85c         14.81abc           6.62a         41.85a         15.89a           6.30ab         38.70b         14.95ab           6.23ab         38.39bc         13.59c           6.00b         37.64bc         14.08bc           6.60a         38.00bc         14.27bc

Note: There is no statistically significant difference between means with the same letter(s) in a column when using LSD ( $p \le 0.05$ ).

# Effect of Ca on SPAD chlorophyll content

There was no interaction between the treatments and the concentration of chlorophyll throughout the course of the weeks, as measured by the SPAD value at  $p \ge 0.05$  (Table 2). However, the increase in chlorophyll content in all treatments showed an inconsistent trend from two to twelve weeks after the use of different Ca concentrations. At 200 ppm of Ca, the SPAD chlorophyll content increased from 45.46 to 53.08 from week two until twelve. Treatment 1000 ppm of Ca showed that the SPAD chlorophyll content rose from 50.68 to 55.48 from week two until twelve. The SPAD chlorophyll content increased from 44.21 to 47.99 from two to twelve for treatment 2500 ppm of Ca during the study period. For treatment 4000 ppm of Ca, the SPAD chlorophyll content in oil palm seedlings increased from 48.46 to 53.65 from week two until twelve. At 5500 ppm of Ca, the SPAD chlorophyll content was increased from 44.53 to 52.19 from week two until twelve. While treatment 7000 ppm of Ca increased from 47.77 to 53.22 from week two until twelve. These findings demonstrated that

treatment 1000 ppm Ca resulted in significantly higher chlorophyll content in oil palm seedlings than in the control and other treatments.

**Table 2.** SPAD Chlorophyll content of oil palm seedlings treated with varied concentration of Ca at 2, 4, 6-, 8-, 10- and 12-weeks post treatment

Ca	Weeks					
Concentrations (ppm)	2	4	6	8	10	12
200	45.46 <sup>bcd</sup>	47.96 <sup>bc</sup>	49.78 <sup>b</sup>	52.05 <sup>ab</sup>	52.28ª	53.08ª
1,000	50.68 <sup>ba</sup>	51.96ª	54.50 <sup>a</sup>	55.24ª	55.64ª	55.48ª
2,500	44.21 <sup>d</sup>	48.09°	50.00 <sup>ab</sup>	51.98 <sup>ab</sup>	51.18ª	47.99 <sup>b</sup>
4,000	$48.46^{ab}$	51.41 <sup>ab</sup>	51.11 <sup>ab</sup>	52.05 <sup>ab</sup>	52.57ª	53.65
5,500	44.53 <sup>cd</sup>	45.16 <sup>c</sup>	47.73 <sup>b</sup>	51.19 <sup>b</sup>	51.27 <sup>a</sup>	52.19 <sup>ab</sup>
7,000	47.77 <sup>abc</sup>	47.35 <sup>abc</sup>	47.26 <sup>b</sup>	47.58°	51.52ª	53.22ª

Note: There is no statistically significant difference between means with the same letter(s) in a column when using LSD ( $p \le 0.05$ ).

# Effect of Ca on macro-nutrients concentration in oil palm

To explore the nutrient partitioning in oil palm tissues, laboratory analysis was carried out (Tables 3 and 4). The results of this investigation revealed that all Ca treatments led to larger nutrient concentrations being partitioned in the shoot section of the plant as opposed to the root. The examination of primary and secondary macronutrients in the shoot section of oil palm seedlings (Table 3) revealed noticeable variations in nitrogen, phosphorus, potassium, calcium, and magnesium when the seedlings were subjected to varying quantities of calcium, with a significance level of  $p \le 0.05$ . Similar results in a root section showed that all macro-nutrients vary significantly in treatment application at  $p \le 0.05$  (Table 4).

Ca concentrations	Concentrations (%)					
(ppm)	Ν	Р	K	Ca	Mg	
200	1.92 <sup>bc</sup>	0.13 <sup>cd</sup>	2.43ª	0.06 <sup>bc</sup>	0.05 <sup>b</sup>	
1000	1.81°	0.15 <sup>ab</sup>	2.47 <sup>a</sup>	0.08°	0.06 <sup>a</sup>	
2500	2.19 <sup>ab</sup>	$0.14^{cbd}$	2.11 <sup>b</sup>	0.09 <sup>b</sup>	0.05°	
4000	2.24 <sup>a</sup>	0.12 <sup>d</sup>	1.74°	0.14 <sup>a</sup>	0.04 <sup>d</sup>	
5500	1.86 <sup>c</sup>	0.15 <sup>ab</sup>	1.82°	0.15 <sup>a</sup>	0.04 <sup>d</sup>	
7000	1.45 <sup>d</sup>	$0.14^{abc}$	1.88 <sup>c</sup>	$0.07^{\rm bc}$	0.04 <sup>d</sup>	

Table 3. Nutrient concentration of oil palm seedlings at shoot section

Note: There is no statistically significant difference between means with the same letter(s) in a column when using LSD ( $p \le 0.05$ ).

N concentration was found to be higher in the shoot section of oil palm seedlings, according to the findings. It started to decrease as the Ca supplied increased from 5500 to 7000 ppm of Ca in Hoagland solution (Table 3). A similar trend also can be found in the root section of oil palm seedlings which N concentration started to decrease when supplied with an excessive amount of Ca from 2500 to 7000 ppm of Ca in Hoagland solution (Table 4). These findings indicated that an excess supply of Ca will reduce the availability of N.

Ca concentrations (ppm)	1	Con	centrations (	%)	
	Ν	Р	К	Ca	Mg
200	1.92 <sup>bc</sup>	0.13 <sup>cd</sup>	2.43ª	0.06 <sup>bc</sup>	0.05 <sup>b</sup>
1000	1.61ª	0.16 <sup>d</sup>	1.89 <sup>ab</sup>	0.07°	0.03ª
2500	1.61ª	0.65ª	1.64 <sup>cd</sup>	0.23 <sup>b</sup>	0.03ª
4000	1.51 <sup>ab</sup>	0.65ª	1.90 <sup>a</sup>	0.23 <sup>b</sup>	0.02 <sup>b</sup>
5500	1.43 <sup>bc</sup>	0.42 <sup>b</sup>	1.41 <sup>d</sup>	0.23 <sup>b</sup>	0.02°
7000	1.40 <sup>bc</sup>	0.30 <sup>c</sup>	1.44 <sup>cd</sup>	0.30 <sup>a</sup>	0.02 <sup>c</sup>

**Table 4.** Nutrient concentration of oil palm seedlings at root section

Note: There is no statistically significant difference between means with the same letter(s) in a column when using LSD ( $p \le 0.05$ ).

The results showed that P concentration in the shoot section of oil palm seedlings showed an irregular pattern (increased and decreased) when supplied with excessive Ca in Hoagland solution (Table 3). However, the P concentration in the root section of oil palm seedlings decreased when supplied with an excessive Ca, from 4000 ppm to 7000 ppm of Ca in Hoagland solution (Table 4). These findings indicated that an excess supply of Ca completely restricts P's availability. Typically, the findings of the K concentration in either the shoot- or root-sections of oil palm seedlings exhibited an uneven pattern. The potassium concentration in the shoot section of oil palm seedlings for treatment was much lower than the concentrations of 200 ppm, 1000 ppm, and 2500 ppm (Table 3). The concentration in the root section of oil palm seedlings, on the other hand, revealed an inconsistent pattern when the amount of Ca that was provided in the Hoagland solution was increased (Table 4). Though, the K content in oil palm seedlings was more significant than the Ca concentration.

Calcium (Ca) concentration in oil palm seedlings considerably increased when the amount of Ca supplied increased from 1000 ppm to 5500 ppm of Ca except for 7000 ppm of Ca (Table 3). Likewise, the nutrient partitioning result observed a similar trend in the root section of oil palm seedlings (Table 4). The Ca concentration decreased when supplied with an excessive Ca from treatment 2500 ppm to 7000 ppm of Ca. The findings revealed that increasing Ca supply increased Ca availability in oil palm seedlings, decreasing the availability of N, P, and Mg in Hoagland solution, thus inhibiting its uptake into the oil palm seedlings. The shoot section indicated the highest magnesium (Mg) concentration value in oil palm seedlings at treatment 1000 ppm of Ca compared to control and other treatments (Table 3). In the root section of oil palm seedlings (Table 4), magnesium (Mg) concentration was significantly higher when grown in a solution containing treatment 200 ppm and 1000 ppm of Ca. However, Mg concentration decreased when supplied with an excessive Ca from 2500 ppm to 7000 ppm of Ca in Hoagland solution. The results showed that excess Ca reduces nutrient availability in oil palm seedlings to Mg.

# The proportion of macronutrients absorbed by oil palm seedlings relative to Ca

The results presented here clearly showed that the increment of Ca concentration from 200 ppm to 7000 ppm in CaCl<sub>2</sub> would reduce the amount of Mg concentration in oil palm shoot- and root-section. The current study indicates that applying excessive amounts of Ca counteract Mg in shoot- and root-section of plant tissues (Table 5). The ratio of Ca to Mg in the solution culture is seen to match with the concentration of Ca found in oil palm leaves. Even though the absorption was slightly limited by the excessive K, the results indicated that the Ca supply was optimal. However, the excessive K did not suppress an excessive amount of Mg in oil palm tissue.

Section	Ca concentrations (ppm)	Ca/N	Ca/P	Ca/K	Ca/Mg
Shoot	200	20.82	15.32	0.63	1.89
	1,000	30.73	19.82	0.80	1.78
	2,500	28.24	20.64	0.98	2.06
	4,000	30.28	24.68	1.44	2.39
	5,500	38.93	26.68	1.50	2.58
	7,000	21.79	15.34	0.81	2.66
Root	200	22.88	14.34	0.73	3.43
	1,000	225.19	35.05	2.15	3.61
	2,500	284.77	42.73	2.33	4.09
	4,000	132.71	30.70	2.28	4.70
	5,500	128.70	44.21	3.05	4.72
	7,000	170.03	45.73	2.70	4.80

**Table 5.** The proportion of macronutrients absorbed by oil palm seedlings relative to Ca

#### Discussion

# Effect of Ca on total biomass

Total biomass of oil palm seedlings treated with 1000 ppm of Ca indicated that the addition of adequate Ca to the nutrient solution resulted in a substantial increase in total biomass. This could be due to increased availability of other nutrients, thereby improving seedlings' chances of consuming nutrients. In comparison with previous studies, using *Zoysia* grass by Xu *et al.* (2013) between control, 200, 400 and 800 ppm Ca from CaCl<sub>2</sub> obtained that the application of 400 ppm Ca from CaCl<sub>2</sub> pre-treatment group increased in biomass of *Zoysia japonica* (*Zoysia* grass) about 150% more than the control treatment. A similar finding by Amor *et al.* (2010) found that the addition of 140 ppm Ca from CaCl<sub>2</sub> showed a maximal growth potential than the addition of 800 ppm Ca from CaCl<sub>2</sub> in enhancing growth for Cakile maritima which is about 120% of the control value. In this study, oil palm seedlings require a much higher concentration of Ca (1000 ppm Ca) as compared to Zoysia grass and Cakile maritima seeds. These findings suggested that oil palm seedlings have a more extensive root system than Zoysia grass and Cakile maritima seeds. Furthermore, the study revealed that the ability of roots to take up Ca varies between plants and is dependent on the genotypic difference in Ca accumulation between the plants.

This study shows that the ideal concentration of  $CaCl_2$  has a substantial impact on plant development and growth, and that this in turn increases the overall biomass value. In addition, these findings indicated that the absorption of Ca was increased using Ca in water-soluble forms, such as  $CaCl_2$  or calcium nitrate  $[Ca(NO_3)_2]$ . However, the role of accompanying anions could potentially affect the absorption of Ca and the parameters measured. Therefore, it is important to maintain an adequate level of available nutrients through fertilizers or manipulating the supply of nutrients as an integrally essential part of agriculture production (Tayed and Hamdan, 1999).

# Effect of Ca on plant growth parameters

As a result of the current research, the determination of vegetative grown in oil palm seedling correlated with the presence of Ca with different concentrations in a nutrient solution to stay healthy. Adding 1000 ppm of Ca to the nutrient solution significantly enhanced the growth and development of oil palm seedlings, demonstrating the importance of Ca in plant nutrition. This could be due to increased availability of other nutrients, thereby improving seedlings' chances of consuming nutrients.

In comparison with previous research recorded by Jefferies and Willis (1964) showed a maximum amount of Ca in CaCl<sub>2</sub> solution required for the growth of different plant species. They found that the growth of Juncus squarrosus was maximal at 277.5 ppm of Ca, while the concentration content of up to 1,331.8 ppm of Ca for Origanum vulgare and Sieglingia decumbens. A similar finding was reported by Clarkson (1965) which revealed that the maximum value of Ca concentration in Nardus stricta for growth was found at 55.5 ppm. These findings make it abundantly evident that the plant must have access to an adequate amount of Ca through its environment. The fact that the results of the current study differ from those of the prior study should be taken

into consideration because the current study employed a different technique and used different kinds of plants.

The Ca is typically a secondary nutrient essential to crop production. All plants need it in large amounts to form cell walls and membranes. Apart from that, it plays a vital role in soil structure. Most Ca found in plants is found in a molecule known as Ca pectate, which is found in the cell walls (Burstrom, 1968). Due to the immobility of Ca in the nutrient solution, soil, and plant tissues, it is essential for plants to have a constant supply of Ca to access it. In the absence of an adequate concentration of calcium, the cell walls would disintegrate, and the plant would not be able to maintain its upright position (Burstrom, 1968). Because of the importance of nutrient availability to agricultural growth, it was crucial to use fertilizers.

#### Effect of Ca on SPAD chlorophyll content

A pigment called chlorophyll influenced the productivity of plants. The amount of chlorophyll that is present in each region can provide information about the photosynthetic capacity and productivity of plants. According to Onwurah *et al.* (2007), the availability of nutrients and environmental conditions such as drought, heat, and salt can have a favorable impact on the quantity of chlorophyll that is present in the leaf tissues of plants. According to the findings of this experiment, the quantity of chlorophyll content in oil palm seedlings rose whenever they were given varying levels of Ca. This was the case regardless of the amount of calcium that was given to them. In line with the findings of Khayyat *et al.* (2009), which showed that the application of 72 ppm of calcium from CaCl<sub>2</sub> treatment in strawberry plants (*Fragaria* × *ananassa* Duch.), our findings agree with those findings resulting in lower chlorophyll content. Nevertheless, the increment in CaCl<sub>2</sub> concentration from 0.72 to 144 ppm of Ca significantly increased the chlorophyll content in strawberry leaves (0.95 to 1.1 mg g<sup>-1</sup> of fresh weight).

In this study, the optimum Ca concentration rate was larger in oil palm seedlings than the strawberry due to a genotypic difference in Ca uptake between plant species. However, both results showed that the amount of leaf chlorophyll content was increased as supplied with CaCl<sub>2</sub> compared to control. This result, therefore, revealed that the application of Ca in nutrient solution could increase the amount of chlorophyll content in the seedlings of the oil palm. However, Ca is not known to be directly required at any step-in enzyme reactions leading to chlorophyll synthesis. Perhaps, it is known to regulate and alter the absorption of nitrogen (N) and magnesium (Mg) ions, which are the essential components of chlorophyll (Laloraya and Pol, 1972).

### Effect of Ca on macro-nutrients concentration in oil palm

The findings of this study indicate that the supplementation of oil palm seedlings with N and K leads to an increase in the concentration of nutrients in the shoot section of the plant. This may be the result of a higher rate of amino acid translocation to the shoot section compared to the root section. Although the oil palm seedlings were developed in solution culture, the Ca/N ratios that they absorbed were lower, particularly in the shoot section of the plant. In oil palm seedlings, the administration of Ca influenced the absorption of N, either in the shoot- or root-section of the plant, which resulted in a shortfall of N. However, the growth of the oil palm became stifled, and the leaves of the seedlings took on a tint that was like a pale green, especially at the base of the pinnae became yellowish or chlorosis due to a degrading of chlorophyll content in leaves. The chlorophyll content was closely linked with N in the leaf since most of the leaf N was contained in chlorophyll molecules. The impact of a plant's Ca state on its later reaction to N was a topic that received a very little amount of attention. Nevertheless, early research conducted by Miillikan et al. (1968) shown that Ca was necessary for the reduction of nitrate  $(NO_3)_2$  in the process of protein synthesis in plants. These findings supported the existence of Ca-N interactions.

For P nutrient concentration, both P shoot- or root-section concentration was lower than Ca. There also seemed to be an antagonism between Ca and P  $(Ca \times P)$  uptake in oil palm seedlings. A high Ca content may be necessary to facilitate the transfer of oil palm seedlings from their roots to their shoots and to increase the shoots' physical robustness. Contrarily, it is not out of the question that steady Ca absorption and a high concentration are incompatible. Using a high concentration of Ca in the nutrient solution, our research was able to encourage a high concentration of Ca in oil palm seedlings. On the other hand, a high concentration of Ca in the nutrient solution also has the potential to cause precipitation of P at the roots of the plant. A similar conclusion was reported by Jakobsen (1979), who stated that their research suggested that the concentration of Ca ions at the surfaces of barley roots might precipitate P, which would then prevent the plant from absorbing not just Ca but also P for a brief period. A further investigation conducted by Jakobsen (1993) shown that the counteracting effect that occurs in the vicinity of nutrient-absorbing roots is produced by the precipitation of calcium phosphates  $[Ca_3(PO_4)_2]$  that are less soluble.

For K concentration of nutrient, both K shoot- or root-section concentration was higher than Ca and Mg. The findings suggested that K was extremely mobile within oil palm seedlings. However, the transport and partitioning of K can vary depending on the source of N, NH<sub>4</sub><sup>+</sup>, or NO<sub>3</sub><sup>-</sup> (Zhang *et al.*, 2010). In the current experiment, oil palm seedlings that were grown in solution culture exhibited decreased Ca/K ratios absorption in most of the treatments, particularly in the shoot section. The only exceptions to this were treatments containing 4000 ppm and 5500 ppm of Ca, which could not be ascribed to solution culture binding

sites. Different root absorption capacities and different levels of calcium transport throughout the plant might be the cause of variations in the Ca/K ratios of different plants. The Ca absorption was more influenced than the K absorption, which makes sense given that the Ca absorption was predominantly dependent on the formation of new roots. According to Chanderbali *et al.* (2013), Ca was mostly transported by the xylem and was dependent on transpiration. However, the root epidermal cells' plasma membranes actively absorbed potassium. Since the potassium (K) did not adhere to the cell wall but rather diffused throughout the cells, especially at the root's central borders, its distribution is independent of the xylem sap's flow rate (Chanderbali *et al.*, 2013).

For hydroponic procedures, the connection between K, Ca, and Mg was critical. When fertilizer salts dissolve in water, they dissociate, releasing nutrients in their charged ionic form. As a result, the nutrients K, Ca, and Mg were positively charged ions or cations with a comparable charge or valence in their ionic state. It is a protein that is embedded in the membrane root of oil palm seedlings that is responsible for the absorption of Ca and K. This protein is responsible for catalyzing the transport of nutrients across the membrane. Because they were both comparable cations, it is possible that they will compete with one another for binding to a particular protein. Because of this, it is possible that an antagonistic response in oil palm seedlings of the Ca  $\times$  K interaction, which is caused by competition at the common plasma membrane transporters (P3A-type H-ATPases), might be relevant to our study (Schulz, 2010). On the other hand, the molecular mechanism of Mg absorption is not well understood; thus, there are no recognized transporters for Mg in the plasma membrane. Thus, too much K nutrition can hinder Ca or Mg nutrient uptake in this study.

However, it will be interesting here to mention the effect of Ca especially on Mg in oil palm seedlings. The results showed that the increment of Ca concentration would reduce the amount of Mg concentration in oil palm shootand root-section. The current study indicates that applying excessive amounts of Ca counteract Mg in shoot- and root-section of plant tissues. The ratio of Ca to Mg in the solution culture is seen to match with the concentration of Ca found in oil palm leaves. Even though the absorption was slightly limited by the excessive K, the results indicated that the Ca supply was optimal. However, the excessive K did not suppress an excessive amount of magnesium in oil palm tissue. Nguyen et al. (2015) discovered that the concentration of Ca in Pummelo (Citrus maxima Merr.) leaves is related to the Ca and Ca/Mg mole proportion in the soil, which is consistent with the findings in this study. The Ca/Mg of Pummelo leaves treated with CaSO<sub>4</sub> together with low Mg in soil tended to be higher than control. However, the Ca/Mg ratios varied in a widely before and after treatment were 3.41 to 3.70, respectively. In agreement with the results of this research, Voogt (1988) also demonstrated that the Ca and Mg ion in beefsteak tomato strongly associated with each other during the uptake process. The high ratio of Ca to Mg in the soil makes it difficult for Mg to be absorbed, which results in a Mg deficit (Bergmann, 1992; Nguyen *et al.*, 2015). The absorption of Ca was shown to be more rapid when its concentration was raised, even when the soil contained a low percentage of magnesium. According to the findings of a study that was conducted in the past by Hao and Papadopoulos (2003), it has been mentioned that the Ca shortage in tomatoes has been supplemented by raising the concentration of Mg in a solution that contains a low amount of Ca.

The concentration of nutrients in plant tissue depends on the absorption, root and shoot biomass, root-to-shoot of nutrient translocation potential, and the rate of transpiration (Ma *et al.*, 2007). Ca given at high concentrations can compensate for even more exceeding 10% of dry weight, such as in mature leaves, with no symptoms of toxicity or significant plant growth restriction (Marschner, 2012). It would be interesting to mention the effect of Ca on other nutrient concentrations, particularly on Mg of oil palm seedlings. Generally, the Ca and Mg ions are essential nutrients for oil palm seedlings. However, these nutrients are highly antagonistic. In case of a higher concentration of one element, the other component will be inhibited (Nguyen *et al.*, 2015). These findings suggested that addition of Ca affected nutritional status of oil palm seedlings especially on Ca and Mg.

Nutrient uptake and availability impacted oil palm seedlings' growth performance, biomass accumulation, and partitioning. Based on this study's findings, better nutrient management is necessary because Ca's nutritional components are essential to the growth and development of oil palm seedlings. Fertilization aims to supply the growing plant with all the nutrients it needs for healthy growth. It is concluded that 1000 ppm provided the best biomass accumulation, nutrient uptake, and growth performance, hence, signifying the optimal value of Hoagland solution for oil palm seedling growth in solution culture. It was advised that the maximum and ideal growth of oil palm seedlings be maintained for experimental purpose. Additional studies are required to identify the effects of different Ca sources on oil palm seedlings' vegetative development, with the optimal concentration of Ca being 1000 ppm.

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