



UNIVERSITI PUTRA MALAYSIA

***NITROGEN UPTAKE AND USE BY OIL PALM SEEDLINGS OF VARIOUS
GENOTYPES AND GENE EXPRESSION OF A NITROGEN RESPONSIVE
GENOTYPE***

LAW CHU CHIEN

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By

LAW CHU CHIEN

**Thesis Submitted to the School of Graduate Studies,
Universiti Putra Malaysia, in Fulfilment of the
Requirements for the Degree of Doctor of Philosophy**

May 2016



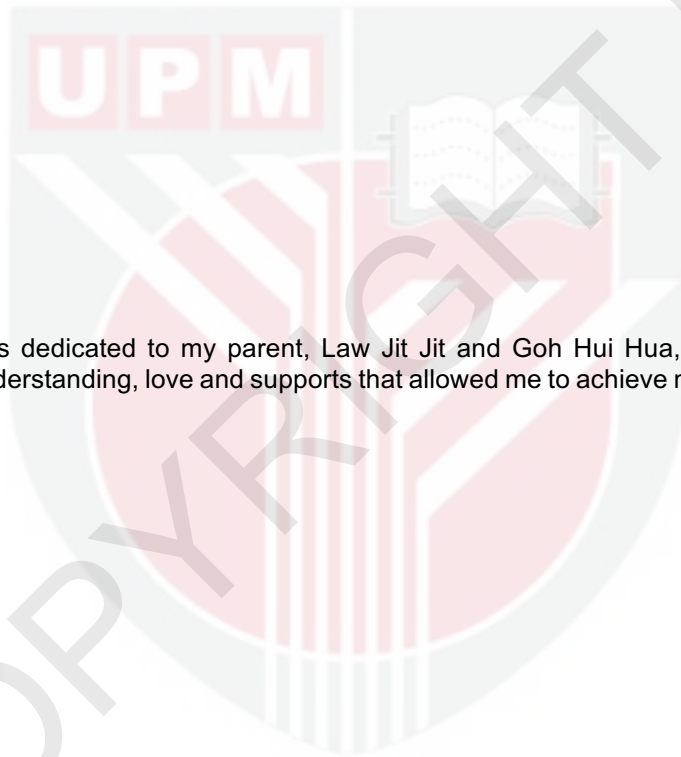
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DEDICATION

This thesis is dedicated to my parent, Law Jit Jit and Goh Hui Hua, for their patience, understanding, love and supports that allowed me to achieve my goals.



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in
fulfilment of the requirement for the degree of Doctor of Philosophy

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By

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May 2016

Chairman: Professor Zaharah Abdul Rahman, PhD

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High economic value of palm oil had gathered much interest for rapid expansion of oil palm plantation in Malaysia. However, most of the soils in Malaysia were generally low in fertility status, hence planting oil palm in these soils could cause further soil degradation and require high Nitrogen (N) fertilizer input in order to maintain high yield output. While fertilizers being the most expensive inputs, increase of application rate would result in increase in production cost as well as introducing negative impact to the environment. To maintain high yield oil palm output with limited soil fertility, effort had been focused on improving oil palm N use efficiencies (NUE). This 3-parts research explore the potential genotypic different of 9 oil palm genotypes which originated from the same high yielding siblings. In part-1, the N uptake performance among selected high yielding oil palm genotypes were quantified. Six-months old and nine-months old oil palm seedlings consist of nine different high yielding genotypes from Sime Darby were cultivated in the glasshouse in a randomized complete block design in split-plots arrangement with four replicates. These oil palms were supplied with ammonium sulphate with 5% atom excess (a.e.) N-15. Destructive sampling was then carried out after 3 months of planting and plant samples were analyzed for total N and N-15 enrichment. Results demonstrated that oil palm at 6 months old did not show any significant difference in N uptake between genotypes either with or without P fertilizer application. However, oil palm at 9 months old demonstrated significant differences between genotypes in total dry matter production and total N uptake. Hence, there was a significant difference in N derived from fertilizer among genotypes. Additionally, oil palms at 9 months old also showed a significant increase in N uptake with P fertilizer application. Genotype A (14/34 × 2367/17) demonstrated significantly higher N uptake compared to the other genotypes, except for genotype F (9/103 × 2318/17). Part-2 of this study was aimed to determine the respond of the oil palm during recovery from N stress condition. Another set of same oil palm genotypes were cultivated and modified Cooper solution (without N) was applied to the oil palm genotypes for 6 months until oil palm leaves turned yellow. After that, standard

Cooper solution was applied to the oil palm genotypes. The SPAD reading were taken daily from frond number three and leaf samples were harvested from frond number three, with interval of 7 days until SPAD reading is stabilized, for total N analysis. All genotypes, with the exception of Genotype I, demonstrated a 9-day lag period before the SPAD reading and the N concentration in the leaves begin to increase. The SPAD reading for Genotype I remained stagnant throughout the whole experimental period. Genotype A showed the fastest recovery ability as compared to the other genotypes. The SPAD readings of the oil palm showed a significant positive association ($r = 0.7337$) with leaf N concentration with leaf N concentration. Part-3 of this study was to determine the gene expression of superior oil palm prior to N stress condition. Genotype A that exhibits favorable positive response to N was subjected to gene expression analysis study. RNA were extracted from root samples collected during the palms were subjected to N-stress and non N-stress conditions. The root RNA samples were then analyzed using microarray analysis. A total of 105,072 gene probes were used in the microarray and 3 genes that related to oil palm N stress respond were identified to be significantly regulated ($p < 0.05$; Fold Changes > 1.5 folds): (1) 9-cis-epoxycarotenoid dioxygenase 1, (2) Early nodulin-93, and (3) Cryptochrome-1. Overall, this research demonstrated that genotypic different among oil palms would affect the plant growth and performance. In particular, Genotype A (14/34 \times 2367/17) used in this study carries had exhibited superiority in N-up taking capability and N-stress tolerance capability. Stagnant period of plant N status prior to N recovery shall also be given attention, as it could greatly alter the management of fertilizer application in oil palm plantations. Genotype A carries the potential gene that could unlock the barrier to breed oil palm genotype which likely to be both high yielding and high NUE. Therefore, further study need to be carried out in order to annotate all possible genes responsible for high NUE, consecutively crossing this gene to the other high yielding oil palm genotypes.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

**PENGAMBILAN DAN PENGGUNAAN NITROGEN OLEH BEBERAPA
GENOTIP BENIH KELAPA SAWIT SERTA EKSPEKSI GEN BAGI GENOTIP
YANG RESPONSIF KEPADA NITROGEN**

Oleh

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Kelapa sawit yang mempunyai nilai ekonomi yang tinggi telah menyebabkan peningkatan keluasan ladang kelapa sawit yang berleluasa di Malaysia. Akan tetapi, kebanyakan tanah di Malaysia adalah kurang memuaskan dari segi kesuburan tanah, maka untuk menanam kelapa sawit di atas tanah sedemikian boleh merangsang degradasi tanah, dan kemudian menyebabkan input baja Nitrogen (N) yang banyak diperlukan untuk memastikan keluaran kelapa sawit terjamin. Baja merupakan input yang paling mahal di ladang kelapa sawit, penambahan kadar pembajaan akan meningkatkan kos pengeluaran dan pada masa yang sama membawa impak negatif kepada alam sekitar. Jadi, bagi memastikan pengeluaran minyak kelapa sawit boleh dioptimumkan pada tanah yang kurang subur, kajian-kajian telah dijalankan bagi meningkatkan kadar kecekapan penggunaan N oleh kelapa sawit. Kajian ini merangkumi tiga bahagian untuk menerokai potensi genotip kelapa sawit yang berlainan yang telah dihasilkan daripada hasil kacukan kelapa sawit yang berhasil tinggi. Dalam bahagian pertama kajian ini, prestasi kadar pengambilan N pokok kelapa sawit antara beberapa genotip yang berhasil tinggi telah ditentukan. Sebanyak 9 genotip benih kelapa sawit yang berhasil tinggi telah didapati daripada syarikat Sime Darby. Anak benih tersebut yang berusia 6 dan 9 bulan telah ditanam di dalam rumah kaca dengan rekabentuk blok rawak lengkap dalam plot berpisah dengan empat replikasi. Benih kelapa sawit kemudiannya diberikan ammonium sulfat dengan eksen atom 5% N-15. Ujian musnah dijalankan kepada kelapa sawit selepas 3 bulan, dan sampel kelapa sawit itu akan dianalisis untuk jumlah N serta kandungan N-15 dalam tisu tumbuhan. Dalam kajian ini, benih kelapa sawit pada usia 6 bulan tidak menunjukkan sebarang perbezaan yang nyata bagi pengambilan N di antara genotip sama ada dengan aplikasi baja P atau tanpa baja P. Tetapi, benih yang berusia 9 bulan telah menunjukkan perbezaan yang nyata antara genotip dalam jumlah berat kering dan pengambilan N. Oleh sebab itu, ia juga menyebabkan perbezaan yang nyata antara genotip bagi N yang berasal dari baja. Genotip A (14/34 × 2367/17) telah menunjukkan pengambilan N yang jauh lebih tinggi berbanding dengan genotip

yang lain kecuali Genotip F ($9/103 \times 2318/17$). Dalam bahagian kedua kajian ini, reaksi kelapa sawit semasa mengalami pemulihan daripada situasi kekurangan N yang serius akan ditelitikan. Set kelapa sawit yang kedua disediakan di dalam rumah kaca. Cecair Cooper yang telah diubah-suai supaya tidak mengandungi sebarang N telah diberikan kepada benih-benih kelapa sawit selama 6 bulan sehingga daun benih menjadi kuning. Kemudian, cecair Cooper yang biasa diberikan kepada semua genotip kelapa sawit. Maka bacaan SPAD diambil setiap hari daripada pelepah daun ketiga. Sampel daun juga diambil di pelepah ketiga setiap 7 hari bagi menentukan kandungan jumlah N. Proses ini berulang sehingga bacaan SPAD menjadi stabil. Semua genotip kecuali genotip I, telah pun menunjukkan bacaan SPAD dan jumlah N dalam tisu yang pegun pada 9 hari yang bermula sebelum meningkat. Bacaan SPAD bagi genotip I adalah pegun sepanjang masa kajian ini dijalankan walaupun telah diberikan cecair Cooper yang mempunyai N. Dalam kajian ini, genotip A menunjukkan kadar pemulihan yang paling laju berbanding dengan genotip yang lain. Bacaan SPAD bagi semua kelapa sawit mempunyai korelasi yang positive ($r = 0.7337$) dengan kandungan jumlah N dalam tisu daun. Dalam kajian ketiga, ekspresi gen bagi genotip yang unggul dalam keadaan kekurangan N telah ditentukan. Genotip A yang telah menunjukkan sifat-sifat yang memuaskan dalam dua kajian yang sebelum ini telah dipilih untuk menjalankan analisis ekspresi gen. RNA daripada akar benih sawit yang diambil semasa kelapa sawit ini mengalami keadaan kekurangan N dan keadaan normal telah diekstrak. Kemudian RNA akar ini telah dianalisis dengan "Microarray". Sebanyak 105,072 gen siasatan telah digunakan dalam array ini. Sebanyak 3 gen yang berkaitan dengan pengambilan N kelapa sawit telah dikenalpastikan nyata dengan kebarangkalian $p < 0.05$ dan perubahan ekspresi melebihi 1.5 kali ganda, iaitu gen : (1) 9-cis-epoxycarotenoid dioxygenase 1, (2) Early nodulin-93, and (3) Cryptochrome-1. Secara keseluruhannya, kajian ini telah menunjukkan bahawa genotip kelapa sawit yang berlainan boleh membawa impak kepada prestasi pertumbuhan dan sifat kelapa sawit yang berlainan. Dalam kajian ini, Genotip A ($14/34 \times 2367/17$) telah menunjukkan sifat yang unggul dalam pengambilan N dan kebolehan yang baik untuk menghadapi keadaan kurang N. Peringkat pegun bagi jumlah N dalam tisu tumbuhan sempena baja N diberikan selepas kekurangan N patut diberi perhatian kerana ia boleh merangsang aktiviti-aktiviti penaburan baja di ladang kelapa sawit. Genotip A mengandungi gen yang berpotensi untuk menghasilkan kacukan genotip yang mengeluarkan hasil tinggi dan pada masa yang sama boleh menggunakan N dengan lebih cekap. Kajian lanjutan seharusnya dijalankan bagi mengenalpastikan semua gen-gen yang bertanggungjawab bagi meningkatkan kecekapan penggunaan N dan seterusnya menghasilkan kelapa sawit yang berhasil tinggi dan cekap menggunakan N.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

ABA	Absciscic acid
ANOVA	Analysis of variance
AS	Ammonium sulphate
AV	<i>Algemeene Vereniging van Rubberplaters ter Oostkust van Sumatra</i>
BD	Banting Dura
C	Carbon
Ca	Calcium
cDNA	Complementary deoxyribonucleic acid
CEC	Cation exchange capacity
CHATS	Constitutive high affinity transport system
CM	Chlorophyll meter
CPO	Crude palm oil
cRNA	Complementary ribonucleic acid
DM	Dry matter
D×P	<i>Dura × Pisifera</i>
FFB	Fresh fruit bunches
ha	Hectares
HATS	High affinity transport system
IAEA	International Atomic Energy Agency
IHATS	Inducible high affinity transporters
JL	Johore Labis
K	Potassium
K _m	Substrate affinity
LATS	Constitutive low affinity transporters
Mg	Magnesium
MINT	Malaysian Institute for Nuclear Technology Research
MPOB	Malaysian Palm Oil Board
mRNA	Messenger ribonucleic acid
N	Nitrogen
Ndff	Nitrogen derived from fertilizer
NH ₃	Ammonia
NH ₄ ⁺	Ammonium
NO ₂	Nitrogen dioxide
N ₂ O	Nitrous oxide
NO ₃ ⁻	Nitrate
NRC	National Research Council
NUE	Nitrogen use efficiencies
P	Phosphorus

p	Probability
qPCR	Quantitative real-time polymerase chain reaction
RCBD	Randomized complete block design
RNA	Ribonucleic acid
Rb	Rubidium
SAS	Statistical Analysis System
SDS-PAGE	Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis
SPAD	Soil Plant Analysis Development
USDA	United States Department of Agriculture
UR	Ulu Remis
V_{\max}	Maximum flux





CHAPTER 1

INTRODUCTION

1.1 Background

Elaeis guineensis Jacq., which is the name given to oil palm by Nicholas Joseph Jacquin in 1763, originated from the tropical rainforest region of West Africa. The oil palm is grown on the same land continuously for 25 to 30 years per cropping cycle and therefore, per ha cropland, oil palm plantations give more oil than any other temperate or tropical oil crop (Corley and Tinker, 2003). The oil palm by far is the most efficient oil crop and alone is capable to fulfill the large and growing world demand for vegetable oils that is estimated to reach 240 million t by year 2050 (Corley, 2009). It produces an average oil yield of $3.68 \text{ t ha}^{-1} \text{ yr}^{-1}$ which is ten times higher than that of soybean, almost nine times that of sunflower seed, and six times that of rapeseed (Basiron, 2007). There are two types of vegetable oil extracted from the palm fruit on commercial scale, i.e. crude palm oil and kernel palm oil. These palm oils have different fatty acid profiles and thus increase the versatility of oil palm in industrial applications. In addition, many researches had been conducted in order to develop numerous ways for the palm oils and the oil palm tree itself to be utilized to form varieties of end products. With reference to its so many positive attributes together with the increasing of global demand for oils and fats, the oil palm industries contribute significantly to Malaysia's National Gross Export (Azman *et al.*, 2004). Thereby, the oil palm had become the most important industrial cash crop in Malaysia.

More and more lands are currently being planted with oil palm in order to increase the oil palm production. The total oil palm area in 2014 was 5.39 million ha, an increase of 3.1% as compared to the previous year. The total export of oil palm products, consisting of palm oil, palm kernel oil, palm kernel cake, oleochemicals, biodiesel and finished products declined by 2.5% to 25.07 million t in 2014 from 25.70 million t recorded in 2013. However, total export revenue increased by 3.7% to RM63.62 billion compared to the RM61.36 billion achieved in 2013 due to higher export prices (Choo, 2015). This further provokes the expansion of oil palm plantations in Malaysia. Rapid increases of oil palm plantation had caused more land were developed as oil palm plantation. However, most of the soils in Malaysia planted to oil palm are from the order Ultisols and Oxisols, which are low in fertility status (Goh *et al.*, 2003), and planting of oil palm in these soils could cause further soil degradation (Tessens and Shamshuddin, 1983). Applying additional fertilizers to compensate nutrient removal from soils by plant seems to be a direct answer to this situation. Therefore, oil palm becomes the largest fertilizer consuming crop in Malaysia.

Oil palm cultivation requires a significant amount of fertilizer for optimum production. Malaysian Palm Oil Board (MPOB) (2009) reported that the total import value of fertilizers for Malaysia from 2004 to 2006 averaged about RM

2.50 billion yearly. A fraction of total fertilizer imports, urea recorded improvement from RM 282 millions at 2004 to RM 525 million at 2008. Total Nitrogen (N) fertilizer consumed in Malaysia industrial plantation recorded 1.29 million t in 2008 (Sabri, 2009). Due to the high fertilizer prices and large planting hectareage of oil palm plantation, fertilizer inevitably becomes most expensive inputs in oil palm production. It accounts for 50-70% of field operational cost and approximately 25% of the total cost of production (Goh and Hardter, 2003). However, the nutrient requirements of oil palm vary widely, depending on the target yield, genetic potential of the planting material used, and numerous environmental factors, such as palm spacing, palm age, soil fertility, ground cover conditions, and climate (Tarmizi and Mohd Tayeb, 2006; Goh and Hardter, 2003). The total annual nutrient uptake of mature oil palms was about 192, 11, 209, 71 and 36 $\text{ha}^{-1} \text{yr}^{-1}$ for N, phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg), respectively. The estimate nutrients removed by oil palm in producing 25 t ha^{-1} of fresh fruit bunches (FFB) were 73, 5.1, 78, 14 and 12 kg $\text{ha}^{-1} \text{yr}^{-1}$ for N, P, K, Ca, Mg, respectively (Goh and Hardter, 2003). Nevertheless, due to highly weathered soil characteristic in Malaysia, *i.e.* mostly Ultisols and Oxisols, most of these fertilizers often suffer from leaching losses and does not become available to oil palms after application especially during high precipitation season. Therefore, more fertilizers inputs were required to compensate nutrient lost and maintain high yield output.

The perception of an increase in fertilization, especially N, always results in increased yield, has led to excessive application of N fertilizers. This practice causes harmful consequences to 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 N fertilizers applied could be loss at the end of the planting season (Sukreeyapongse *et al.*, 2001). Therefore, increasing fertilizer rates without proper precaution often leads to intense environmental pollution as well as significant economical loss. A study by Goh (2005) also indicated that over-application of as much as 0.25 kg ammonium nitrate palm⁻¹ yr⁻¹ will result in an extra cost of RM 117.25 million per year to the oil palm industry in Malaysia. Hence, overcoming nutrient loss from soils through increasing fertilizer application was apparently economically unfavorable. From the other point of view, reducing fertilizer rate as an initiative to prevent environment pollution does not solve the problem effectively. Insufficient nutrient supply to meet the requirement of oil palm could result in significant drop in productivity of oil palm for the subsequent years (Mohd Tayeb and 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. Additionally, Goh (2005) reported that under-application of fertilizers might result in loss of oil palm yield. Hence, increasing oil palm N use efficiency becomes crucial in optimizing N use, maintaining high yield rates, reducing fertilizer cost, and avoid N fertilizer pollutions in order to ensure an environmentally friendly and sustainable oil palm management.

There are several literatures regarding genetic materials of oil palm, as well as N fertilizer uptake on oil palm. However, quantification of oil palm N uptake

performance through genetic selection is still at the infancy stage. Nitrogen transporter gene expression of superior oil palm prior to N stress condition also inadequately studied. Information regarding physiological and molecular respond of oil palm in N stress condition as compared to oil palm in non-stressed condition is still lacking.

1.2 Research Objectives

The objectives of the study were:

- (i) to quantify oil palm N uptake performance among selected high yielding genotype;
- (ii) to determine the response of oil palm during recovery from N stress; and
- (iii) to determine the gene expression of oil palm grown with and without N stress conditions.

BIBLIOGRAPHY

- Aldrich, S. R. (1980). *Nitrogen, in Relation to Food, Environment, and Energy*. Illinois Agricultural Experiment Station Special Publication 61. Urbana-Champaign: University of Illinois.
- Allanach, K., Mengel, M., Einecke, G., Sis, B., Hidalgo, L. G., Mueller, T., and Halloran, P. F. (2008). Comparing microarray versus RT-PCR assessment of renal allograft biopsies: similar performance despite different dynamic ranges. *American Journal of Transplantation*, 8(5), 1006–1015.
- Alves, B. J. R., Resende, A. S., Urquiaga, S., and Boddey, R. M. (2000). Biological nitrogen fixation by two tropical forage legumes assessed from the relative ureide abundance of stem solutes: ^{15}N calibration of the technique in sand culture. *Nutrient Cycling in Agroecosystems*, 56(2), 165–176.
- Aslam, M., Travis, R. L., and Huffaker, R. C. (1992). Comparative kinetics and reciprocal inhibition of nitrate and nitrite uptake in roots of uninduced and induced barley seedlings. *Plant Physiology*, (99), 1124–1133.
- Azman, I., Esnan, A. G., and Kushairi, A. (2004). Kesesuaian tanaman sawit. In A. G. Esnan, Z. Z. Zin, and W. Mohd. Basri (Eds.), *Perusahaan Sawit di Malaysia - Satu Panduan* (p. 325). Kajang, Selangor: Lembaga Minyak Sawit Malaysia.
- Bai, J., Gao, H., Xiao, R., Wang, J., and Huang, C. (2012). A review of soil nitrogen mineralization as affected by water and salt in coastal wetlands: Issues and methods. *CLEAN - Soil, Air, Water*, 40(10), 1099–1105.
- Balazadeh, S., Schildhauer, J., Araújo, W. L., Munné-Bosch, S., Fernie, A. R., Proost, S., Humbeck, K., and Mueller-Roeber, B. (2014). Reversal of senescence by N resupply to N-starved *Arabidopsis thaliana*: transcriptomic and metabolomic consequences. *Journal of Experimental Botany*, 65(14), 3975–3992.
- Basiron, Y. (2007). Palm oil production through sustainable plantations. *European Journal of Lipid Science and Technology*, 109(4), 289–295.
- Bergstrom, L., and Kirchmann, H. (2004). Leaching and crop uptake of nitrogen from nitrogen-15-labeled green manures and ammonium nitrate. *Journal of Environment Quality*, 33(5), 1786–1792.
- Bernado, R. (2002). *Breeding for Quantitative Traits in Plants*. Woodbury, Minnesota: Stemma Press.
- Bi, Y.-M., Kant, S., Clarke, J., Clark, J., Gidda, S., Ming, F., Xu, J., Rochon, A., Shelp, B. J., Hao, L., Zhao, R., Mullen, R. T., Zhu, T., and Rothstein, S. J. (2009). Increased nitrogen-use efficiency in transgenic rice plants over-expressing a nitrogen-responsive early nodulin gene identified from rice expression profiling. *Plant, Cell and Environment*, 32(12),

1749–1760.

- Bi, Y.-M., Wang, R.-L., Zhu, T., and Rothstein, S. J. (2007). Global transcription profiling reveals differential responses to chronic nitrogen stress and putative nitrogen regulatory components in *Arabidopsis*. *BMC Genomics*, 8, 281.
- Blackmer, A. M. (1999). Bioavailability of nitrogen. In M. E. Sumner (Ed.), *Handbook of Soil Science* (p. D-3-D-18). Boca Raton, FL: CRC Press LLC.
- Brady, N. C., and Weil, R. R. (2003). *Elements of the Nature and Properties of Soils* (2nd ed.). Upper Saddle River, NJ: Prentice Hall, Inc.
- Bremner, J. M., and Mulvaney, C. S. (1982). Nitrogen-total. In A. L. Page, R. H. Miller, and D. R. Keeney (Eds.), *Methods of Soil Analysis. Part 2. Chemical and microbiological properties* (2nd ed., pp. 595–624). Madison, WI: American Society of Agronomy and Soil Science Society of America.
- Britto, D. T., and Kronzucker, H. J. (2006). Plant nitrogen transport and its regulation in changing soil environments. *Journal of Crop Improvement*, 15(2), 1–23.
- Broeshart, H., Ferwerda, J. D., and Kovachich, W. G. (1957). Mineral deficiency symptoms of the oil palm. *Plant and Soil*, 8(4), 289–300.
- Brophy, L., and Heichel, G. (1989). Nitrogen release from roots of alfalfa and soybean grown in sand culture. *Plant and Soil*, 116(1), 77–84.
- Broschat, T. K. (1984). Nutrient deficiency symptoms in five species of palms grown as foliage plants. *Principes*, 28(1), 6–14.
- Campbell, W. H. (1988). Nitrate reductase and its role in nitrate assimilation in plants. *Physiologia Plantarum*, 74(1), 214–219.
- Chakrabarti, N., and Mukherji, S. (2003). Effect of phytohormone pretreatment on nitrogen metabolism in *Vigna radiata* under salt stress. *Biologia Plantarum*, 46(1), 63–66.
- Chalk, P. M. (1985). Estimation of N₂ fixation by isotope dilution: An appraisal of techniques involving ¹⁵N enrichment and their application. *Soil Biology and Biochemistry*, 17(4), 389–410.
- Chalk, P. M. (1996). Estimation of N₂ fixation by ¹⁵N isotope dilution—The A-value approach. *Soil Biology and Biochemistry*, 28(9), 1123–1130.
- Chamuah, G. S. (1988). The effect of nitrogen on root growth and nutrient uptake of young tea plants (*Camellia sinensis* L.) grown in sand culture. *Nutrient Cycling in Agroecosystems*, 16(1), 59–65.
- Chardon, F., Barthelemy, J., Daniel-Vedele, F., and Masclaux-Daubresse, C. (2010). Natural variation of nitrate uptake and nitrogen use efficiency in *Arabidopsis thaliana* cultivated with limiting and ample nitrogen supply. *Journal of Experimental Botany*, 61(9), 2293–2302.

- Chen, Y. Te, Borken, W., Stange, C. F., and Matzner, E. (2012). Dynamics of nitrogen and carbon mineralization in a fen soil following water table fluctuations. *Wetlands*, 32(3), 579–587.
- Choo, Y. M. (2011). Overview of the Malaysian oil palm industry 2011. Retrieved from http://bepi.mpob.gov.my/images/overview/Overview_of_Industry_2011.pdf
- Choo, Y. M. (2015). Overview of the Malaysian oil palm industry 2014. Retrieved from http://bepi.mpob.gov.my/images/overview/Overview_of_Industry_2014.pdf
- Churchill, G. A. (2002). Fundamentals of experimental design for cDNA microarrays. *Nature Genetics*, 32(4s), 490–495.
- Cleland, R. E., Letham, D. S., Goodwin, P. B., and Higgins, T. J. V. (1980). Phytohormones and related compounds: A comprehensive treatise. *BioScience*, 30(2), 123–123.
- Clement, C. R., Hopper, M. J., and Jones, L. H. P. (1978). The uptake of nitrate by *Lolium perenne* from flowing nutrient solution. 1. Effect of nitrate concentration. *Journal of Experimental Botany*, 29(2), 453–464.
- Cooke, J. E. K., Martin, T. A., and Davis, J. M. (2005). Short-term physiological and developmental responses to nitrogen availability in hybrid poplar. *The New Phytologist*, 167(1), 41–52.
- Cooper, A. (1979). *The ABC of NFT*. London, UK: Grower Books.
- Corley, R. H. V. (2009). How much palm oil do we need ? *Environmental Science and Policy*, 12(2), 134–139.
- Corley, R. H. V., and Tinker, P. B. (2003). *The Oil Palm. World Agriculture Series* (4th ed.). Hoboken, NJ: John Wiley and Sons, Inc.
- Corley, R. H. V, Hardon, J. J., and Wood, B. J. (1976). *Oil Palm Research: Developments in Crop Science (1)*. Amsterdam, Netherlands: Elsevier Scientific Publishing Company.
- Crawford, N. M., and Glass, A. D. M. (1998). Molecular and physiological aspects of nitrate uptake in plants. *Trends in Plant Science*, 3(10), 389–395.
- Danso, S. K. A. (1986). Review: Estimation of N₂-fixation by isotope dilution: An appraisal of techniques involving ¹⁵N enrichment and their application--Comments. *Soil Biology and Biochemistry*, 18(3), 243–244.
- Debouba, M., Gouia, H., Suzuki, A., and Ghorbel, M. H. (2006). NaCl stress effects on enzymes involved in nitrogen assimilation pathway in tomato "*Lycopersicon esculentum*" seedlings. *Journal of Plant Physiology*, 163(12), 1247–1258.

- Debouba, M., Maâroufi-Dghimi, H., Suzuki, A., Ghorbel, M. H., and Gouia, H. (2007). Changes in growth and activity of enzymes involved in nitrate reduction and ammonium assimilation in tomato seedlings in response to NaCl stress. *Annals of Botany*, 99(6), 1143–51.
- Draghici, S., Khatri, P., Eklund, A. C., and Szallasi, Z. (2006). Reliability and reproducibility issues in DNA microarray measurements. *Trends in Genetics : TIG*, 22(2), 101–9.
- Errebhi, M., Rosen, C. J., Gupta, S. C., and Birong, D. E. (1998). Potato yield response and nitrate leaching as influenced by nitrogen management. *Agronomy Journal*, 90(1), 10–15.
- Fairhurst, T. (1996). *Management of Nutrients for Efficient Use in Smallholder Oil Palm Plantations*. PhD Thesis, University of London, London, UK.
- Foong, S. F., and Lee, C. T. (1998). Early results on the effect of various sources of phosphate rock on oil palm yield. *Paper for Discussion Agromac. Audit 1998. FELDA Agricultural Services Sdn. Bhd.*
- Forde, B. G. (2000). Nitrate transporters in plants: structure, function and regulation. *Biochimica et Biophysica Acta (BBA) - Biomembranes*, 1465(1), 219–235.
- Forde, B. G., and Clarkson, T. (1999). Nitrate and ammonium nutrition of plants: Physiological and molecular perspectives. *Advances in Botanical Research*, 30, 1–90.
- Frenay, J. R. (2005). Options for reducing the negative effects of nitrogen in agriculture. *Science in China Series C: Life Sciences*, 48(Supplement 2), 861–870.
- Frink, C. R., Waggoner, P. E., and Ausubel, J. H. (1999). Nitrogen fertilizer: Retrospect and prospect. *Proceedings of the National Academy of Sciences of the United States of America*, 96(4), 1175–1180.
- Galitz, D. S. (1979). Uptake and assimilation of nitrogen by plants. *Farm Research*, 37(3), 16–18.
- Gao, H., Bai, J., He, X., Zhao, Q., Lu, Q., and Wang, J. (2014). High temperature and salinity enhance soil nitrogen mineralization in a tidal freshwater marsh. *PloS One*, 9(4), e95011.
- Gillbanks, R. A. (2003). Standard agronomic procedures and practices. In T. H. Fairhurst and R. Hardter (Eds.), *Oil Palm: Management For Large And Sustainable Yields* (pp. 115–150). Singapore: Potash and Phosphate Institute of Canada (PPIC) and International Potash Institute (IPI).
- Glass, A. D. M., Britto, D. T., Kaiser, B. N., Kronzucker, H. J., Kumar, A., Okamoto, M., Rawat, S. R., Siddiqi, M. Y., Silim, S. M., and Vidmar, J. J. (2001). Nitrogen transport in plants, with an emphasis on the regulation of fluxes to match plant demand. *Journal of Plant Nutrition and Soil Science*,

(164), 199–207.

- Glass, A. D. M., and Siddiqi, M. Y. (1995). Nitrogen absorption by plant roots. In H. S. Srivastava and R. P. Singh (Eds.), *Nitrogen Nutrition in Higher Plants* (pp. 21–56). New Delhi, India: Associated Publishing Company.
- Goel, P., and Singh, A. K. (2015). Abiotic stresses downregulate key genes involved in nitrogen uptake and assimilation in brassica juncea l. *PLoS ONE*, 10(11), e0143645.
- Goh, K. J. (2005). Fertilizer recommendation systems for oil palm: Estimating the fertiliser rates. In P. S. Chew and Y. P. Tan (Eds.), *Proceedings of MOSTA Best Practices Workshops - Agronomy and Crop Management* (pp. 235–268). Kuala Lumpur: Malaysian Oil Scientists' and Technologists' Association (MOSTA).
- Goh, K. J., Chew, P. S., and Teo, C. B. (1994). Maximising and maintaining oil palm yields on commercial scale in Malaysia. In K. H. Chee (Ed.), *Management for Enhanced Profitability in Plantations* (pp. 121–141). Kuala Lumpur: Incorporated Society of Planters (ISP).
- Goh, K. J., and Hardter, R. (2003). General oil palm nutrition. In T. Fairhurst and R. Hardter (Eds.), *Oil Palm: Management for Large and Sustainable Yields* (pp. 191–230). Singapore: Potash and Phosphate Institute of Canada (PPIC) and International Potash Institute (IPI).
- Goh, K. J., Hardter, R., and Fairhurst, T. (2003). Fertilizing for maximum return. In T. Fairhurst and R. Hardter (Eds.), *Oil Palm: Management for Large and Sustainable Yields* (pp. 279–306). Singapore: Potash and Phosphate Institute of Canada (PPIC) and International Potash Institute (IPI).
- Gomes Da Silveira, J. A., De Brito Cardoso, B., Barreto De Melo, A. R., and Viégas, R. A. (1999). Salt-induced decrease in nitrate uptake and assimilation in Cowpea plants. *Revista Brasileira de Fisiologia Vegetal*, 11(2), 77–82.
- Gough, N. R. (2009). More roles for cryptochromes. *Science Signaling*, 2(58), ec61–ec61.
- Hamilton, S. D., Chalk, P. M., Smith, C. J., and Hopmans, P. (1991). Effect of N fertilizer rate on the estimation of N₂ fixation by isotope dilution. *Soil Biology and Biochemistry*, 23(12), 1105–1110.
- Hardon, J. J. (1970). Inbreeding in populations of the oil palm (*Elaeis guineensis* Jacq.) and its effect on selection. *Oleagineux*, 25, 449–456.
- Hardon, J. J. (1976). Oil palm breeding - introduction. In R. H. V. Corley, J. J. Hardon, and B. J. Wood (Eds.), *Oil Palm Research* (pp. 89–108). Amsterdam, Netherlands: Elsevier.
- Hardon, J. J., Rao, V., and Rajanaidu, N. (1985). A review of oil palm breeding. In G. E. Russell (Ed.), *Progress in Plant Breeding* (pp. 139–163). London,

UK: Butterworths.

Hardon, J. J., and Thomas, R. L. (1968). Breeding and selection of the oil palm in Malaya. *Oleagineaux*, 3, 85–90.

Hartley, C. W. S. (1988). *The Oil Palm* (3rd ed.). Harlow, UK: Longman.

Hartung, W., Peuke, A., and Davies, W. (1999). Absciscic acid - A hormonal long-distance stress signal in plants under drought and salt stress. In M. Pessarakli (Ed.), *Handbook of Plant and Crop Stress* (2nd ed., pp. 731–747). CRC Press.

Hartung, W., Radin, J. W., and Hendrix, D. L. (1988). Absciscic acid movement into the apoplastic solution of water-stressed cotton leaves: Role of apoplastic pH. *Plant Physiology*, 86(3), 908–913.

Hazelton, P. A., and Murphy, B. W. (2007). *Interpreting Soil Test Results: What Do All the Numbers Mean?* (2nd ed.). Collingwood, Australia: CSIRO Publishing.

Hellemans, J., Mortier, G., De Paepe, A., Speleman, F., and Vandesompele, J. (2007). qBase relative quantification framework and software for management and automated analysis of real-time quantitative PCR data. *Genome Biology*, 8(2), R19.

Huez-López, M. A., Ulery, A. L., Samani, Z., Picchioni, G., and Flynn, R. P. (2011). Response of Chile pepper (*Capsicum annuum* L.) to salt stress and organic and inorganic nitrogen sources: II. Nitrogen and water use efficiencies, and salt tolerance, 14(3), 757–763.

IAEA. (1975). *Root Activity Patterns of Some Tree Crops. A joint undertaking by the IAEA and FAO. Technical reports series No. 170*. Vienna, Austria: International Atomic Energy Agency (IAEA).

IAEA. (2001). *Use of Isotope and Radiation Methods in Soil and Water Management and Crop Nutrition. Training course series No. 14*. Vienna, Austria: International Atomic Energy Agency (IAEA).

Islam, M. S., Bhuiya, M. S. U., Rahman, S., and Hussain, M. (2009). Evaluation of SPAD and LCC based nitrogen management in rice (*Oryza sativa* L.). *Bangladesh Journal of Agricultural Research*, 34(4), 661–672.

Ito, S., Hara, T., Kawanami, Y., Watanabe, T., Thiraporn, K., Ohtake, N., Sueyoshi, K., Mitsui, T., Fukuyama, T., Takahashi, Y., Sato, T., Sato, A., and Ohya, T. (2009). Carbon and nitrogen transport during grain filling in rice under high-temperature conditions. *Journal of Agronomy and Crop Science*, 195(5), 368–376.

Jourdan, C., and Rey, H. (1997a). Modelling and simulation of the architecture and development of the oil-palm (*Elaeis guineensis* Jacq.) root system. I. The model. *Plant and Soil*, 190(2), 217–233.

- Jourdan, C., and Rey, H. (1997b). Modelling and simulation of the architecture and development of the oil-palm (*Elaeis guineensis* Jacq.) root system. II. Estimation of root parameters using the RACINES postprocessor. *Plant and Soil*, 190(2), 235–246.
- Justic, D., Rabalais, N. N., Turner, R. E., and Dortch, Q. (1995). Changes in nutrient structure of river-dominated coastal waters: stoichiometric nutrient balance and its consequences. *Estuarine, Coastal and Shelf Science*, 40(3), 339–356.
- Kee, K. K., and Chew, P. S. (1996). Nutrient losses through surface runoff and soil erosion – Implications for improved fertiliser efficiency in mature oil palms. In A. Darus, M. B. Wahid, N. Rajanaidu, H. T. Dolmat, K. Paranjothy, S. C. Cheah, K. C. Chang, and S. Ravigadevi (Eds.), *1996 PORIM International Palm Oil Congress: Competitiveness for the 21st Century*, Kuala Lumpur (pp. 153–169). Kuala Lumpur: Palm Oil Research institute of Malaysia.
- Khan, M. G., and Srivastava, H. S. (1998). Changes in growth and nitrogen assimilation in maize plants induced by NaCl and growth regulators. *Biologia Plantarum*, 41(1), 93–99.
- King, B. J., Siddiqi, M. Y., Ruth, T. J., Warner, R. L., and Glass, A. D. M. (1993). Feedback regulation of nitrate influx in barley roots by nitrate, nitrite and ammonium. *Plant Physiology*, 102(4), 1279–1286.
- Kirda, C., Schepers, J. S., Derici, M. R., and Schepers, J. S. (2001). Yield response and N-fertilizer recovery of rainfed wheat growing in the Mediterranean region. *Field Crops Research*, 71(2), 113–122.
- Kushairi, A., Rajanaidu, N., Jalani, B. S., and Zakri, A. H. (1999). Agronomic performance and genetic variability of Dura x Pisifera progenies. *Journal of Oil Palm Research*, 11(2), 1–24.
- Landis, T. D., Haase, D. L., and Dumroese, R. K. (2005). Plant nutrient testing and analysis in forest and conservation nurseries. In R. K. Dumroese, L. E. Riley, T. D. Landis, and tech. coords. 2005. (Eds.), *National proceedings: Forest and Conservation Nursery Associations—2004; 2004 July 12–15; Charleston, NC; and 2004 July 26–29; Medford, OR. Proc. RMRS-P-35* (pp. 76–83). Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station.
- Law, C. C., Zaharah, A. R., Husni, M. H. A., and Siti Nor Akmar, A. (2012). Evaluation of nitrogen uptake efficiency of different oil palm genotypes using ¹⁵N isotope labelling method. *Pertanika Journal of Tropical Agricultural Science*, 35(4), 744–754.
- Law, C. C., Zaharah, A. R., Husni, M. H. A., and Siti Nor Akmar, A. (2014). Leaf nitrogen content in oil palm seedlings and their relationship to SPAD chlorophyll meter readings. *Journal of Oil Palm, Environment and Health*, 5, 8–17.

- Lewis, D. (1951). The metabolism of nitrate and nitrite in sheep. 1. The reduction of nitrate in the rumen of the sheep. *Biochemical Journal*, 48(2), 175–180.
- Li, Q. H., and Yang, H. Q. (2007). Cryptochrome signaling in plants. *Photochemistry and Photobiology*, 83(1), 94–101.
- Lian, X., Wang, S., Zhang, J., Feng, Q., Zhang, L., Fan, D., Li, X., Yuan, D., Han, B., and Zhang, Q. (2006). Expression profiles of 10,422 genes at early stage of low nitrogen stress in rice assayed using a cDNA microarray. *Plant Molecular Biology*, 60(5), 617–631.
- Luo, Z. Bin, Calfapietra, C., Liberloo, M., Scarascia-Mugnozza, G., and Polle, A. (2006). Carbon partitioning to mobile and structural fractions in poplar wood under elevated CO₂ (EUROFACE) and N fertilization. *Global Change Biology*, 12(2), 272–283.
- Maene, L. M., Thong, K. C., Ong, T. S., and Mokhtaruddin, A. M. (1979). Surface wash under mature oil palm. In E. Pushparajah (Ed.), *Symposium on Water in Malaysian Agriculture, Kuala Lumpur* (pp. 203–216). Kuala Lumpur: Malaysian Society of Soil Science (MSSS).
- Mansouri, A., and Lurie, A. A. (1993). Concise review: methemoglobinemia. *American Journal of Hematology*, 42(1), 7–12.
- Masclaux-Daubresse, C., Daniel-Vedele, F., Dechorgnat, J., Chardon, F., Gaufichon, L., and Suzuki, A. (2010). Nitrogen uptake, assimilation and remobilization in plants: challenges for sustainable and productive agriculture. *Annals of Botany*, 105(7), 1141–57.
- Meharg, A. A., and Blatt, M. R. (1995). Nitrate transport across the plasma membrane of *Arabidopsis thaliana* root hairs: Kinetic control by pH and membrane voltage. *Journal of Membrane Biology*, 145(1), 49–66.
- Mengel, K. (1992). Nitrogen: agricultural productivity and environmental problems. In K. Mengel and D. J. Pilbeam (Eds.), *Nitrogen Metabolism of Plants (Proceedings of the Phytochemical Society of Europe 33)* (p. 289). New York: Oxford University Press.
- Mifflin, B. J., and Lea, P. J. (1977). Amino acid metabolism. *Annual Review of Plant Physiology*, 28, 299–329.
- Miller, A. J., and Smith, S. J. (1996). Nitrate transport and compartmentation in cereal root cells. *Journal of Experimental Botany*, 47(7), 843–854.
- Min, X. J., Siddiqi, M. Y., Guy, R. D., Glass, A. D. M., and Kronzucker, H. J. (2000). A comparative kinetic analysis of nitrate and ammonium influx in two early-successional tree species of temperate and boreal forest ecosystems. *Plant, Cell and Environment*, 23(3), 321–328.
- Mohammed Hashim, A. T., and Mohd Ali, S. (2006). Impact of mineral petroleum prices on Fertilizers. *The Planter*, 82(968), 727–728.

- Mohd Noor, M., Mohd Arif, S., Azman, I., and Jusoh, L. (2005). Palm oil costs of production for Malaysia: Trend and performance. *The Planter*, 81(957), 745–762.
- Mohd Tayeb, D., and Tarmizi, A. M. (2001). Manuring philosophy in the midst of declining palm oil prices. In *Proceedings of the National Seminar on Positioning the Malaysian Palm Oil Industry in Challenging Times* (pp. 34–42). Bangi, Selangor: Malaysian Palm Oil Board (MPOB).
- MPOB. (2009). Crop Statistics. Retrieved from www.mpob.gov.my
- National Research Council (NRC). (1978). *Nitrates: An environmental assessment*. Washington, D. C.: National Academy of Science.
- Nelson, D. W., and Sommers, L. E. (1982). Total carbon, organic carbon, and organic matter. In A. L. Page, R. H. Miller, and D. R. Keeney (Eds.), *Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties* (2nd ed., pp. 539–579). Madison, WI: American Society of Agronomy and Soil Science Society of America.
- Ng, S. K., von Uexkull, H., and Hardter, R. (2003). Botanical aspects of the oil palm relevant to crop management. In T. Fairhurst and R. Hardter (Eds.), *Oil Palm: Management for Large and Sustainable Yields* (pp. 13–26). Singapore: Potash and Phosphate Institute of Canada (PPIC) and International Potash Institute (IPI).
- Nichols, R. (1964). Studies on the major-element deficiencies of the pigeon pea (*Cajanus cajan*) in sand culture. *Plant and Soil*, 21(3), 377–387.
- Norziha, A., Rafii, M. Y., Maizura, I., and Ghizan, S. (2008). Genetic variation among oil palm parent genotypes and their progenies based on microsatellite markers. *Journal of Oil Palm Research*, 20, 533–541.
- NRC. (1978). *Nitrates: An environmental assessment*. Washington, D. C.: National Academy of Science.
- Oaks, A., and Hirel, B. (1985). Nitrogen metabolism in roots. *Annual Review of Plant Physiology*, 36(1), 345–365.
- Olsen, S. R., and Sommers, L. E. (1982). Phosphorus. In A. L. Page, R. H. Miller, and D. R. Keeney (Eds.), *Methods of Soil Analysis. Part 2 Chemical and microbiological properties* (2nd ed., pp. 403–430). Madison, WI: American Society of Agronomy and Soil Science Society of America.
- Ooi, S. C., Hardon, J. J., and Phang, S. (1973). Variability in the Deli dura breeding population of the oil palm (*Elaeis guineensis* Jacq). *Malaysian Agricultural Journal*, 49(2), 112–121.
- Peng, M., Bi, Y.-M., Zhu, T., and Rothstein, S. J. (2007). Genome-wide analysis of *Arabidopsis* responsive transcriptome to nitrogen limitation and its regulation by the ubiquitin ligase gene NLA. *Plant Molecular Biology*, 65(6), 775–97.

- Percival, G. C., Keary, I. P., and Noviss, K. (2008). The potential of a chlorophyll content SPAD meter to quantify nutrient stress in foliar tissue of sycamore (*Acer pseudoplatanus*), English oak (*Quercus robur*), and European beech (*Fagus sylvatica*). *Arboriculture and Urban Forestry*, 34(2), 89–100.
- Peuke, A. D., Jeschke, W. D., and Hartung, W. (1994). The uptake and flow of C, N and ions between roots and shoots in *Ricinus communis* L. III. Long-distance transport of abscisic acid depending on nitrogen nutrition and salt stress. *Journal of Experimental Botany*, 45(6), 741–747.
- Pitre, F. E., Pollet, B., Lafarguette, F., Cooke, J. E. K., MacKay, J. J., and Lapierre, C. (2007). Effects of increased nitrogen supply on the lignification of poplar wood. *Journal of Agricultural and Food Chemistry*, 55(25), 10306–14.
- Rabalais, N., Turner, R., Justić, D., Dortch, Q., Wiseman, W., and Sen Gupta, B. (1996). Nutrient changes in the Mississippi River and system responses on the adjacent continental shelf. *Estuaries*, 19(2), 386–407.
- Rafii, M. Y., Rajanaidu, N., Jalani, B. S., and Kushairi, A. (2002). Performance and heritability estimations on oil palm progenies tested in different environments. *Journal of Oil Palm Research*, 14(1), 15–24.
- Rajanaidu, N., and Rao, V. (1988). Oil palm genetic collections: their performance and use to the industry. In A. H. Hassan, P. S. Chew, B. J. Wood, and E. Pushparajah (Eds.), *Proceedings of the 1987 International Oil Palm Conference - Agriculture* (pp. 59–85). Kuala Lumpur: Palm Oil Research institute of Malaysia (PORIM).
- Rawat, S. R., Silim, S. N., Kronzucker, H. J., Siddiqi, M. Y., and Glass, A. D. M. (1999). AtAMT1 gene expression and NH_4^+ uptake in roots of *Arabidopsis thaliana*: Evidence for regulation by root glutamine levels. *Plant Journal*, 19(2), 143–152.
- Rennenberg, H., Wildhagen, H., and Ehlting, B. (2010). Nitrogen nutrition of poplar trees. *Plant Biology*, 12(2), 275–291.
- Riley, W. J., Ortiz-Monasterio, I., and Matson, P. A. (2001). Nitrogen leaching and soil nitrate, nitrite, and ammonium levels under irrigated wheat in Northern Mexico. *Nutrient Cycling in Agroecosystems*, 61(3), 223–236.
- Ritchie, M. E., Diyagama, D., Neilson, J., van Laar, R., Dobrovic, A., Holloway, A., and Smyth, G. K. (2006). Empirical array quality weights in the analysis of microarray data. *BMC Bioinformatics*, 7(1), 261.
- Ritchie, M. E., Silver, J., Oshlack, A., Holmes, M., Diyagama, D., Holloway, A., and Smyth, G. K. (2007). A comparison of background correction methods for two-colour microarrays. *Bioinformatics*, 23(20), 2700–2707.
- Rosenquist, E. A. (1986). The genetic base of oil palm breeding populations. In A. C. Soh, N. Rajanaidu, and M. Nasir (Eds.), *Proceedings of the International Workshop on Oil Palm Germplasm and Utilization* (pp. 16–27).

Kuala Lumpur: Palm Oil Research institute of Malaysia (PORIM).

- Russow, R., and Böhme, F. (2005). Determination of the total nitrogen deposition by the ^{15}N isotope dilution method and problems in extrapolating results to field scale. *Geoderma*, 127(1–2), 62–70.
- Sabri, M. A. (2009). Evolution of fertilizer use by crops in Malaysia: Recent trends and prospects. In *Proceedings of the International Fertilizer Industry Association (IFA) Crossroads Asia-Pacific 2009 Conference, Kota Kinabalu, Malaysia, 8-10 December 2009*. Paris, France: IFA.
- Sawyer, J. E., Lundvall, J., Hawkins, J. S., and Barker, D. W. (2011). *Sensing Nitrogen Stress in Corn*. Ames, Iowa: Iowa State University.
- Sexton, B. T., Moncrief, J. F., Rosen, C. J., Gupta, S. C., and Cheng, H. H. (1996). Optimizing nitrogen and irrigation inputs for corn based on nitrate leaching and yield on a coarse-textured soil. *Journal of Environment Quality*, 25(5), 982–992.
- Sexton, P., and Carroll, J. (2002). Comparison of SPAD chlorophyll meter readings vs. petiole nitrate concentration in sugarbeet. *Journal of Plant Nutrition*, 25(9), 1975–1986.
- Sharma, P., Chatterjee, M., Burman, N., and Khurana, J. P. (2014). Cryptochrome 1 regulates growth and development in Brassica through alteration in the expression of genes involved in light, phytohormone and stress signalling. *Plant, Cell and Environment*, 37(4), 961–77.
- Siddiqi, M. Y., Glass, A. D. M., Ruth, T. J., and Rufty, T. W. (1990). Studies of the uptake of nitrate in barley. *Plant Physiology*, 93(4), 1426–1432.
- Simmonds, N. W. (1979). *Principles of Crop Improvement*. London, UK: Longman.
- Smyth, G. K. (2005). Limma: Linear models for microarray data. In R. Gentleman, V. Carey, S. Dudoit, R. Irizarry, and W. Huber (Eds.), *Bioinformatics and Computational Biology Solutions Using R and Bioconductor* (pp. 397–420). New York: Springer.
- Soh, A. C. (2004). Selecting the ideal oil palm: What you see is not necessarily what you get. *Journal of Oil Palm Research*, 16(2), 121–128.
- Soh, A. C., and Tan, S. T. (1983). Estimation of genetic variance, heritability and combining ability in oil palm breeding. In T. C. Yap, K. M. Graham, and Jalani Sukaimi (Eds.), *Proceedings of the Fourth International SABRAO Congress on Crops Improvement Research* (pp. 379–388). Bangi, Selangor: Society for the Advancement of Breeding Researches in Asia and Oceania (SABRAO).
- Sukreeyapongse, O., Panichsakpatana, S., and Thongmarg, J. (2001). Nitrogen leaching from soil treated with sludge. *Water Science and Technology*, 44(7), 145–150.

- Tahir, I. S. A., and Nakata, N. (2005). Remobilization of nitrogen and carbohydrate from stems of bread wheat in response to heat stress during grain filling. *Journal of Agronomy and Crop Science*, 191(2), 106–115.
- Tan, K. H. (2005). *Soil Sampling, Preparation, and Analysis* (2nd ed.). Boca Raton, Florida: CRC Press.
- Tarmizi, A. M., and Mohd Tayeb, D. (2006). Nutrient demands of tenera oil palm planted on inland soil of Malaysia. *Journal of Oil Palm Research*, 18, 204–209.
- Tessens, E., and Shamshuddin, J. (1983). *Quantitative Relationships between Mineralogy and Properties of Tropical Soils*. Serdang, Selangor, Malaysia: Penerbit Universiti Pertanian Malaysia.
- Thomas, G. W. (1982). Exchangeable cations. In A. L. Page, R. H. Miller, and D. R. Keeney (Eds.), *Methods of Soil Analysis. Part 2 Chemical and microbiological properties* (2nd ed., pp. 159–165). Madison, WI: American Society of Agronomy and Soil Science Society of America.
- Thomas, R. L., Watson, I., and Hardon, J. J. (1969). Inheritance of some components of yield in the “deli dura variety” of oil palm. *Euphytica*, 18(1), 92–100.
- Tinker, P. B., and Leigh, R. A. (1985). Nutrient uptake by plants - efficiency and control. In A. T. Bachik and E. Pushparajah (Eds.), *Proceedings of the International Conference on Soils and Nutrition of Perennial Crops* (pp. 3–17). Kuala Lumpur: Malaysian Society of Soil Science (MSSS).
- Tremblay, N., Wang, Z., and Bélec, C. (2007). Evaluation of the dualox for the assessment of corn nitrogen status. *Journal of Plant Nutrition*, 30(9), 1355–1369.
- USDA. (2016). Production, supply and distribution online. Retrieved January 15, 2016, from <http://apps.fas.usda.gov/psdonline/psdHome.aspx>
- von Wiren, N., Gazzarrini, S., Gojon, A., and Frommer, W. B. (2000). The molecular physiology of ammonium uptake and retrieval. *Current Opinion in Plant Biology*, 3, 254–261.
- Waddell, J. T., Gupta, S. C., Moncrief, J. F., Rosen, C. J., and Steele, D. D. (2000). Irrigation- and nitrogen-management impacts on nitrate leaching under potato. *Journal of Environment Quality*, 29(1), 251–261.
- Wang, M. Y., Siddiqi, M. Y., Ruth, T. J., and Glass, A. D. M. (1993). Ammonium uptake by rice roots (II. Kinetics of ammonium influx across the plasmalemma). *Plant Physiology*, 103(4), 1259–1267.
- Wang, R., Guegler, K., LaBrie, S. T., and Crawford, N. M. (2000). Genomic analysis of a nutrient response in *Arabidopsis* reveals diverse expression patterns and novel metabolic and potential regulatory genes induced by nitrate. *The Plant Cell*, 12(8), 1491–1509.

- Wang, R., Okamoto, M., Xing, X., and Crawford, N. M. (2003). Microarray analysis of the nitrate response in *Arabidopsis* roots and shoots reveals over 1,000 rapidly responding genes and new linkages to glucose, trehalose-6-phosphate, iron, and sulfate metabolism. *Plant Physiology*, 132(2), 556–67.
- Wang, Y. H., Garvin, D. F., and Kochian, L. V. (2001). Nitrate-induced genes in tomato roots. Array analysis reveals novel genes that may play a role in nitrogen nutrition. *Plant Physiology*, 127(1), 345–359.
- Wilkinson, S. R., Grunes, D. L., and Sumner, M. E. (1999). Nutrient interactions in soil and plant nutrition. In M. E. Sumner (Ed.), *Handbook of Soil Science* (p. D-89-D-112). Boca Raton, FL: CRC Press LLC.
- Wolt, J. D. (1994). *Soil Solution Chemistry: Applications to Environmental Science and Agriculture*. New York, USA: John Wiley & Sons.
- Xu, P., Xiang, Y., Zhu, H., Xu, H., Zhang, Z., Zhang, C., Zhang, L., and Ma, Z. (2009). Wheat cryptochromes: subcellular localization and involvement in photomorphogenesis and osmotic stress responses. *Plant Physiology*, 149(2), 760–74.
- Yeap, W.-C., Loo, J., Wong, Y., and Kulaveerasingam, H. (2014). Evaluation of suitable reference genes for qRT-PCR gene expression normalization in reproductive, vegetative tissues and during fruit development in oil palm. *Plant Cell, Tissue and Organ Culture*, 116(1), 55–66.
- Yuen, T., Wurmbach, E., Pfeffer, R. L., Ebersole, B. J., and Sealfon, S. C. (2002). Accuracy and calibration of commercial oligonucleotide and custom cDNA microarrays. *Nucleic Acids Research*, 30(10), e48.
- Zaharah, A. R., Sharifuddin, H. A. H., Ahmad Sahali, M., and Mohd Hussein, M. S. (1989). Fertilizer placement studies in mature oil palm using isotope technique. *The Planter*, 65, 284–388.
- Zeevaart, J. A. D., and Creelman, R. A. (1988). Metabolism and physiology of abscisic acid. *Annual Review of Plant Physiology and Plant Molecular Biology*, 39(1), 439–473.
- Zin, Z. Z., Zulkifli, H., and Tarmizi, A. M. (2007). Maximizing the potential of phosphate fertilizers for increasing mature oil palm yield. *MPOB Information Series No. 347. June 2007*.
- Zvomuya, F., Rosen, C. J., Russelle, M. P., and Gupta, S. C. (2003). Nitrate leaching and nitrogen recovery following application of polyolefin-coated urea to potato. *Journal of Environmental Quality*, 32(2), 480–489.