UNIVERSITI PUTRA MALAYSIA

IDENTIFICATION OF SEQUENCE VARIANTS IN KEY VITAMIN E GENES FROM Elaeis guineensis Jacq. GERMPLASM FOR DEVELOPMENT OF DNA-BASED MARKERS

BABURA SULAIMAN RUFA'I

FP 2018 75

IDENTIFICATION OF SEQUENCE VARIANTS IN KEY VITAMIN E GENES FROM *Elaeis guineensis* Jacq. GERMPLASM FOR DEVELOPMENT OF DNA-BASED MARKERS

By

BABURA SULAIMAN RUFA'I

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

February 2018

COPYRIGHT

This thesis is dedicated to my loves ones, especially to my parents, wife, children (Aisha and Asma'u) and all my family members who have given me enormous support and prayers since the beginning of my studies.

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

IDENTIFICATION OF SEQUENCE VARIANTS IN KEY VITAMIN E GENES FROM *Elaeis guineensis* Jacq. GERMPLASM FOR DEVELOPMENT OF DNA-BASED MARKERS

By

BABURA SULAIMAN RUFA'I

February 2018

Chairman: Professor Datin Siti Nor Akmar Abdullah
Faculty: Agriculture

Vitamin E possesses important nutritional attributes that play various roles in human disease protection. The most well-known function of this noble compound is that of chain breaking antioxidant activity that scavenge free radical ions and reduce lipid peroxidation in membrane systems. Homogentisate geranylgeranyl transferase (HGGT) and homogentisate phytyl transferase (HPT) that catalyse the first committed step of tocotrienol and tocopherol biosynthesis, respectively are important in determining plant vitamin E composition. In *Elaeis guineensis*, there is high variability in the level of vitamin E among the germplasm materials from Angolan and Tanzanian origins. Therefore, the first objective of this study was to determine important sequence variants in these key vitamin E genes from *E. guineensis* germplasm materials that can be used for the development of DNA-based markers. The second objective was to analyse the effects of the sequence variants on vitamin E content and composition by overexpression of the *HGGT* gene and its mutant derivatives in *Arabidopsis thaliana*. Sequence analysis reveals no important variants in *HPT* gene that could be associated with low and high vitamin E content. However, the analysis reveals four SNPs at positions 193, 2225, 2429 and 6932 in the coding region of the *HGGT* gene that are associated with the vitamin E content. SNPs at 193 and 2429 positions lead to non-conservative amino acid changes in the sequence from Proline (CCT) in low vitamin E to Serine (TCT) in high vitamin E and from Methionine (ATG) in low to Isoleucine (ATA) in high vitamin E palms, respectively. SNP markers 193F/413R and 2225F/2429R were developed at these SNP locations for selection of high and low vitamin E germplasm materials in *E. guineensis*. Fourty one germplasm materials with different vitamin E level were screened to validate these two functional SNP markers using designed PCR-based mismatch primers. The results showed 100% success of the SNP-based markers in differentiating low and high vitamin E accessions. Furthermore, single nucleotide mutagenesis was successfully carried out to generate three cDNA sequence variants (*193SNPHGGT, 2429SNPHGGT* and *HighSNPHGGT*) with one or both SNP variants incorporated into the sequence of the commercial D×P genotype (*LowSNPHGGT*). The variant *HGGT* cDNA sequences together with the unmodified cDNA were successfully transformed into *Arabidopsis*

thaliana. The relative expression levels of *HGGT* in T_3 homozygous lines having the four different constructs separately showed significant ($P \leq 0.005$) up-regulated expression compared with untransformed wild type *Arabidopsis*. However, there was no significant difference observed in the expression among transgenic *Arabidopsis* plants harbouring the different *HGGT* constructs. This demonstrated that the different variants of the *E. guineensis HGGT* gene was expressed at about the same levels in the transgenic *Arabidopsis*. HPLC analysis indicates significant increase ($p \leq 0.05$) in the total tocotrienol content between wild type and all the four transgenic lines (1.50 – 1.82-fold increase). Similarly, significant difference ($p \leq 0.05$) in total tocotrienol was also recorded within the transgenic lines specifically between the two lines that harboured the two SNPs changes (*HighSNPHGGT)* and the one harboring the unmodified gene (*LowSNPHGGT*), which showed 1.22-fold increase. According to these results the two SNP variants introduced into the *HGGT* sequence of low vitamin E commercial variety affect the tocotrienol content and composition when analysed by functional characterization in *Arabidopsis thaliana*.

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

PENGENALAN VARIAN JUJUKAN DALAM GEN UTAMA VITAMIN E DARIPADA BAHAN GERMPLASMA *Elaeis guineensis* Jacq. UNTUK PEMBANGUNAN PENANDA BERASASKAN DNA

Oleh

BABURA SULAIMAN RUFA'I

Februari 2018

Pengerusi: Profesor Datin Siti Nor Akmar Abdullah, PhD
Fakulti: Pertanian

Vitamin E mempunyai ciri nutrisi penting yang memainkan pelbagai peranan dalam ketahanan penyakit manusia. Fungsi yang paling dikenali bagi sebatian adi ini adalah aktiviti antioksidan pemutus rantai yang mengaut ion radikal bebas dan mengurangkan pemperoksidaan lipid dalam sistem membran. Homogentisat geranilgeranil transferase dan homogentisat fitil transferase yang memangkin langkah berkomitmen pertama daripada biosintesis tokotrienol dan tokoferol adalah penting dalam menentukan komposisi vitamin E tumbuhan. Dalam *Elaeis guineensis*, terdapat variabiliti yang tinggi dalam paras vitamin E di antara bahan germplasma yang berasal dari Angola dan Tanzania. Dengan itu, objektif pertama kajian adalah untuk menentukan varian jujukan penting dalam gen vitamin E utama dari bahan germplasma *E. guineensis* yang boleh digunakan untuk pembangunan penanda berasasakan DNA. Objektif kedua adalah untuk menganalisis kesan varian jujukan keatas kandungan dan komposisi vitamin E melalui pengekspresan melampau gen HGGT dan mutan yang terhasil darinya dalam *Arabidopsis thaliana*. Analysis jujukan mendedahkan tiada varian dengan fungsi yang penting dalam gen *HPT* yang boleh dikaitkan dengan kandungan vitamin E yang rendah dan tinggi. Walau bagaimanapun, analisis mendedahkan empat SNPs pada kedudukan 193, 2225, 2429 dan 6932 dalam kawasan pengekodan gen *HGGT* yang dikaitkan dengan kandungan vitamin E. SNPs pada kedudukan 193 dan 2429, masing-masing membawa kepada perubahan non-konservatif asid amino dalam jujukan dari Prolin (CCT) dalam vitamin E yang rendah kepada Serine (TCT) dalam vitamin E yang tinggi dan dari Methionein (ATG) dari yang rendah kepada Isoleusin (ATA) dalam palma yang bervitamin E tinggi. Penanda SNPs 193F/413R dan 2225F/2429R telah dibangunkan pada lokasi SNPs untuk pemilihan bahan germplasma vitamin E yang tinggi dan rendah dalam *E.guineensis*. Empat puluh satu bahan germplasma dengan paras vitamin E yang berbeza telah disaring untuk mengesahkan dua penanda SNP fungsional menggunakan primer berasasakan PCR yang tidak sepadan. Keputusan menunjukkan 100% kejayaan penanda SNP tersebut dalam membezakan aksesi vitamin E yang rendah dan tinggi. Tambahan pula, kaedah mutagenesis nukleotida tunggal telah berjaya dilakukan untuk menjana tiga varian jujukan cDNA (*193SNPHGGT, 2429SNPHGGT* dan

HighSNPHGGT) dengan satu atau dua varian SNP dimasukkan ke dalam jujukan cDNA genotip D×P komersial (*LowSNPHGGT*). Varian jujukan cDNA bersama dengan cDNA yang tidak diubah suai telah bejaya ditransformkan kedalam *Arabidopsis thaliana*. Paras ekspresi relatif *HGGT* dalam titisan homozigot T_3 yang mempunyai empat konstruk berbeza yang berasingan menunjukkan ekspresi menaik yang ketara ($P \leq 0.05$) berbanding *Arabidopsis* jenis liar yang tidak diubahsuai. Walau bagaimanapun, tiada perbezaan ketara yang diperhatikan dalam ekspresi antara tumbuhan *Arabidopsis* transgenik yang memiliki konstruk *HGGT* yang berbeza. Ini menunjukkan varian gen *HGGT E. guineensis* yang berbeza telah diekspres pada paras yang sama dalam *Arabidopsis* transgenik. Analisis HPLC menunjukkan peningkatan yang ketara ($P \leq 0.05$) dalam kandungan tokotrienol keseluruhan antara jenis liar dan empat titisan transgenik (1.5-1.82 kali ganda meningkat). Begitu juga, perbezaan ketara ($P \leq 0.05$) dalam keseluruhan tokotrienol juga direkodkan diantara titisan transgenik terutama di antara dua titisan yang mempunyai dua perubahan SNPs (*HighSNPHGGT*) dan yang mempunyai gen tidak diubahsuai (*LowSNPHGGT*), yang mana menunjukkan peningkatan 1.22 kali ganda. Berdasarkan penemuan ini, kedua varian SNPs yang dimasukkan kedalam *HGGT* varieti komersial dengan vitamin E rendah memberi kesan terhadap kandungan dan komposisi vitamin E apabila dianalisis melalui pencirian kefungsian dalam *Arabidopsis thaliana*.

ACKNOWLEDGEMENTS

Alhamdulillah, I would like to express my utmost appreciation to my supervisor, Professor Datin Dr. Siti Nor Akmar Abdullah, for her great support, excellence guidance, constructive comments and motivation not only in making the completion of this thesis a reality, but for also making me a better researcher and scholar.

I would like to thank my employee, Bayero University, Kano Nigeria for nominating me to receive scholarship award from Tertiary Education Trust Fund (Tetfund).

I would also like to express my deepest thanks to my beloved wife, for her endless kindness, support and love, infinite patience and understanding, and my dearest children Aisha (Yaya Mama) and Asma'u (Husna).

I will like to express my appreciation to my lab mates, both staff and students, Mrs Rozila Abdulrahman, Miss Siti Radziah, Mr Mehdi, Usama bin Zaid, Mei Yee, Azzreena, Hannan, Shafika, Nazri, Rezique, Hazwan, Enkgo, Abbas and Isyaku who made my life easy in the laboratory.

Finally, very special thanks to my father, mother, Brothers and Sisters for their endless prayer, love and support, and sincere thanks to all of my family members and friends.

I certify that a Thesis Examination Committee has met on 6 February 2018 to conduct the final examination of Babura Sulaiman Rufa'i on his thesis entitled "Identification of Sequence Variants in Key Vitamin E Genes from *Elaeis guineensis* Jacq. Germplasm for Development of DNA-Based Markers" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

Members of the Thesis Examination Committee were as follows:

Wong Mui Yun, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Ho Chai Ling, PhD
Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Internal Examiner)

Mohd Rafii bin Yusop, PhD
Professor
Institute of Tropical Agriculture and Food Security
Universiti Putra Malaysia
(Internal Examiner)

Zoe A Wilson, PhD
Professor
University of Nottingham
United Kingdom
(External Examiner)

RUSLI HAJI ABDULLAH, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 30 July 2018

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

Siti Nor Akmar Abdullah, PhD
Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Halimi B Muhammad Saud, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

Noor Azmi Shaharuddin, PhD
Senior Lecturer
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

ROBIAH BINTI YUNUS, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

Declaration by graduate student

I hereby confirm that:

- this thesis is my original work;
- quotations, illustrations and citations have been duly referenced;
- this thesis has not been submitted previously or concurrently for any other degree at any other institutions;
- intellectual property from the thesis and copyright of thesis are fully-owned by Universiti Putra Malaysia, as according to the Universiti Putra Malaysia (Research) Rules 2012;
- written permission must be obtained from supervisor and the office of Deputy Vice-Chancellor (Research and Innovation) before thesis is published (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials as stated in the Universiti Putra Malaysia (Research) Rules 2012;
- there is no plagiarism or data falsification/fabrication in the thesis, and scholarly integrity is upheld as according to the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) and the Universiti Putra Malaysia (Research) Rules 2012. The thesis has undergone plagiarism detection software.

Signature: _____ Date: _____

Name and Matric No.: Babura Sulaiman Rufa'i, (GS41318)

Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision,
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

Signature: _____
Name of Chairman of Supervisory Committee: Professor Dr. Siti Nor Akmar Abdullah

Signature: _____
Name of Member of Supervisory Committee: Associate Professor Dr. Halimi B Muhammad Saud

Signature: _____
Name of Member of Supervisory Committee: Associate Professor Dr. Noor Azmi Shaharuddin

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

µl	Micro-liter
A.thaliana	*Arabidopsis thaliana*
A.tumefaciens	*Agrobacterium tumefaciens*
ANOVA	Analysis of variance
BLAST	Basic Local Alignment Search Tool
bp	Base Pairs
CaMV	Cauliflower Mosaic Virus
cDNA	Complementary DNA
CDS	Coding Region
CTAB	Hexacetyltrimethyl Ammonium Bromide
D x P	Dura x Pesifera
DEPC	Diethyl Pyrocarbonate
DLD-1	Colorectal anticancer agent
DNA	Deoxyribonucleic Acid
DNase	Deoxyribonuclease
$dNTP_S$	Deoxynucleotides
EDTA	Ethylenediaminetetraacetic acid
EgHGGT	Elaeis guineensis HGGT
EtBr	Ethidium Bromide
FFB	Fresh fruit bunches
Gb	Giga bite
GBS	Genotyping by sequencing
GGDP	Geranylgeranyl diphosphate
GWAS	Genome-wide association study
HGA	Homogentisic acid
HGGT	Homogentisate geranylgeranyl transferase
HPLC	High performance liquid chromatography
HPPD	Hydroxyphenyl pyruvate dioxygenase
HPT	Homogentisate petyltransferase
InDels	Insertion Deletion
KASP	Competitive allele specific PCR
kb	Kilo Base-Pair
Km	Michaelis constant
LB	Luria-Bertani
LD	Linkage disequilibrium
LiAc	Lithium Acetate
LiCl	Lithium Chloride
MAS	Marker-assisted selection
MPOB	Malaysian palm oil board
mRNA	Messenger RNA
MYB	Myeloblastosis gene
NaCl	Sodium Chloride
NaOH	Natrium Hydroxide
NCBI	National Center For Biotechnology Information
ng	Nanogram
NGS	Next-generation sequencing
OD	Optical Density

OFR	Open reading frame
PacBio	Pacific Bioscience
PCR	Polymerase Chain Reactions
PDP	Phytyl diphosphate
pH	potential of hydrogen
PTGs	Post transcriptional gene silencing
PVP	Polyvinylpyrrolidone
qPCR	Quantitative real-time PCR
QTLs	Quantitative trait loci
RNA	Ribonucleic Acid
RNase	Ribonuclease
rpm	Round per minute
rtITP	reversibly terminated deoxyinosine triphosphate
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
S.O.C	Super Optimal Broth
SDS	Sodium Dodecyl Sulphate
SGS	Second generation sequencing
SMRT	Single-molecule real-time
SNP	Single nucleotide polymorphism
SSRs	Simple sequence repeats
TAE	Tris-acetate EDTA
TE buffer	Tris-EDTA buffer
TGS	Third generation sequencing
Tm	Melting temperature
UTR	Untranslated region
UV	Ultraviolent
w.a.a	Week After Anthesis
WT	Wild Type
193SNPHGGT	*HGGT* with mutated SNP at 193 position
2429SNPHGGT	*HGGT* with mutated SNP at 2429 position
HighSNPHGGT	*HGGT* with two mutated SNPs at 193 and 2429 position
LowSNPHGGT	Non Mutated *HGGT*

CHAPTER 1

INTRODUCTION

The term vitamin E is used to describe eight lipophilic, naturally occurring compounds that include four tocopherols designated as α-, β-, γ- δ- tocopherols and their four corresponding tocotrienols (Peh et al., 2015). Tocopherols and tocotrienols isomers are distinguished based on the number and position of the methyl groups on their chromanol ring. Tocopherols have saturated phytyl tail that differentiates them from tocotrienols, which possess unsaturated tail containing three double bonds. Tocopherols are important lipid soluble antioxidants that protect cell membrane from oxidation by reacting with lipid radicals produced in the lipid peroxidation chain reaction (Hunter and Cahoon, 2007). Like tocopherols, tocotrienols are good antioxidants that tend to guard plant cells against any biochemical stress especially those arising from the breakdown of unsaturated fatty acids is the seeds (Das et al., 2005). Compared with tocopherols, tocotrienols are sparsely studied, but the current research direction is starting to give more attention to the tocotrienols, the lesser known but more potent antioxidant in vitamin E. Tocotrienols are believed to possess greater ability than tocopherols in scavenging free radical ions and reducing peroxidation of lipids in membrane system (Shahidi et al., 2010). In addition, some studies suggested that tocotrienols have specialized role in protecting neurons from damage (neurodegradation) (Sen et al., 2006) and cholesterol reduction properties (Das et al., 2005). Oral consumption of tocotrienols protects against stroke-associated brain damage *in vivo* (Khanna et al., 2005). Generally, most reports on vitamin E have shown that many of the properties in tocotrienols are not present in tocopherols. Tocotrienols are commercially produced from extracts of rice and oil palm and can be purchased in different forms (Cahoon et al., 2003).

Crude red palm oil is a unique vegetable oil obtained from the fruits of oil palm tree (*Elaeis guineensis*). The only natural oil produces a mixture of different antioxidants and phytonutrients such as tocopherol/tocotreinol (vitamin E), alpha and beta-carotene (pro-vitamin A) in high level, phytosterol complex and coenzymes. No other vegetable oil has this natural combinations of phytonutrients (Corley, 2007). Malaysian Palm Oil Board (MPOB) has most member of *Elaeis guineensis* germplasm materials in the whole world (Zaki et al., 2012). Among the *E. guineensis* germplasm materials, the Angolan and Tanzanian materials were observed to have high variability in the level of vitamin E content ranging from 300 – 1600 ppm, while the level is 500 – 1000 ppm in the commercial (D×P) variety (Wahid et al., 2005). This variation may arise from the molecular set up of the genes responsible for the production of vitamin E in the plant. Thus, it would be interesting to study functional nucleotide variants in the key vitamin E biosynthetic genes (*HGGT* and *HPT*), which catalyses the first committed step of tocotreinol and tocopherol biosynthesis, respectively.

Identification of potentially functionally important sequence variants in the form of SNPs or indels as well as functional analysis of *HGGT* responsible for tocotrienol biosynthesis

and *HPT* gene responsible for tocopherol biosynthesis in oil palm would be very valuable. It could help in identifying the sources of vitamin E content variation among the accessions, and in the production of nutritionally rich palm oil in the long term. Since such studies have not been done so far, the results obtained will serve as valuable background information for genetic improvement of the oil palm. The objectives of this study are:

1) To determine the sequence variations in *homogentisate geranylgeranyl transferase* (*HGGT*) and *homogentisate phytyltransferase* (*HPT*) genes that differentiates accessions producing high and low vitamin E in *Elaeis guineensis* germplasm materials.
2) To develop SNPs/Indels markers for high and low vitamin E germplasm materials identification based on potential functional nucleotide variants.
3) To produce different expression vector constructs by introducing different combination of variant nucleotides into the *HGGT* sequence from D×P variety for functional studies.
4) To analyse the effects of sequences variant on vitamin E composition and content by overexpression of the *HGGT* gene and its mutant derivatives in transgenic model plant (*Arabidopsis thaliana)*.

REFERENCES

Abbasi, A. R., Saur, A., Hennig, P., Tschiersch, H., Hajirezaei, M., Hofius, D., Sonnewald, U., Voll, L.M., 2009. Tocopherol deficiency in transgenic tobacco (*Nicotiana tabacum L.*) plants leads to accelerated senescence. *Plant Cell Environment*, 32: 144–57.

Abdullah, N., Rafii Yusop M., Saleh, G., Latif M.A., 2011. Genetic variability of oil palm parental genotypes and performance of its progenies as revealed by molecular markers and quantitative traits. *Comptes Rendus Biologies,* 334: 290-299.

Agarwal, M., Shrivastava, N., Padh, H., 2008. Advances in molecular marker techniques and their applications in plant sciences. *Plant Cell Report.* 27: 617–631.

Aggarwal, B.B., Sundaram, C., Prasad, S., Kannappan, R., 2010. Tocotrienols, the vitamin E of the 21st century: Its potential against cancer and other chronic diseases. *Biochemical. Pharmacology,* 30: 1613-1631.

Ahsan, H., Ahad, A., Siddiqui, W.A., 2015. A review of characterization of tocotrienols from plant oils and foods. *Journal of Chemistry and Biology*, 8: 45–59.

Andersen, J.R., Lübberstedt, T., 2003. Functional markers in plants. *Trends in Plant Science*, 8: 554–560.

Arabidopsis Genome Initiative, 2000. Analysis of the genome sequence of the Fowering plant *Arabidopsis thaliana*. *Nature,* 408: 796–815.

Arif, I.A., Bakir, M.A., Khan, H.A., Al Farhan, A.H., Al Homaidan, A.A., Bahkali, A.H., Al Sadoon, M., Shobrak, M., 2010. A brief review of molecular techniques to assess plant diversity. *International. Journal of. Molecular Science,* 11: 2079–2096.

Asensi-Fabado, M.A., Munné-Bosch, S., 2010. Vitamins in plants: occurrence, biosynthesis and antioxidant function. *Trends in Plant Science*, 15: 582–592.

Atanassov, I.I., Etchells, J.P., Turner, S.R., 2009. A simple, flexible and efficient PCR-fusion/Gateway cloning procedure for gene fusion, site-directed mutagenesis, short sequence insertion and domain deletions and swaps. *Plants Methods,* 5:14

Barcelos, E., Rios, S. De, A., Cunha, R.N.V., Lopes, R., Motoike, S.Y., Babiychuk, E., Skirycz, A., Kushnir, S., 2015. Oil palm natural diversity and the potential for yield improvement. *Frontiers in Plant Science*, 6: 190.

Baud, S., Mendoza, M.S., To, A., Harscoët, E., Lepiniec, L., Dubreucq, B., 2007. WRINKLED1 specifies the regulatory action of LEAFY COTYLEDON2 towards fatty acid metabolism during seed maturation in *Arabidopsis*. *Plant Journal*, 50: 825–838.

Berendzen, K., Searle, I., Ravenscroft, D., Koncz, C., Batschauer, A., Coupland, B., Somssich, I.E., Ulker, B., 2005. A rapid and versatile combined DNA/RNA extraction protocol and its application to the analysis of a novel DNA marker set polymorphic between *Arabidopsis thaliana* ecotypes Col-0 and *Landsberg Erecta*. *Plant Methods,* 1: 340–55.

Berlin, K., Koren, S., Chin, C.-S., Drake, J.P., Landolin, J.M., Phillippy, A.M., 2015. Assembling large genomes with single-molecule sequencing and locality-sensitive hashing. *Nature Biotechnology*, 33: 623–630.

Brown, S.D., Nagaraju, S., Utturkar, S., De Tissera, S., Segovia, S., Mitchell, W., Land, M.L., Dassanayake, A., Köpke, M., 2014. Comparison of single-molecule sequencing and hybrid approaches for finishing the genome of *Clostridium autoethanogenum* and analysis of CRISPR systems in industrial relevant Clostridia. *Biotechnology and Biofuels*, 7: 40.

Bui, M., Liu, Z., 2009. Simple allele-discriminating PCR for cost-effective and rapid genotyping and mapping. *Plant Methods*, 5: 1.

Cahoon, E.B., Dietrich, C.R., Meyer, K., Damude, H.G., Dyer, J.M., Kinney, A.J., 2006. Conjugated fatty acids accumulate to high levels in phospholipids of metabolically engineered soybean and *Arabidopsis* seeds. *Phytochemistry*, 67: 1166–1176.

Cahoon, E.B., Hall, S.E., Ripp, K.G., Ganzke, T.S., Hitz, W.D., Coughlan, S.J., 2003. Metabolic redesign of vitamin E biosynthesis in plants for tocotrienol production and increased antioxidant content. *Nature Biotechnology*, 21: 1082–1087.

Che, P., Zhao, Z., Glassman, K., Dolde, D., Hu, T.X., Jones, T.J., Obukosia, S., Wambugu, F., Che, P., Zhao, Z., Glassman, K., Dolde, D., Hu, T.X., Jones, T.J., Fred, D., 2016. Elevated vitamin E content improves all *trans* β-carotene accumulation and stability in biofortified sorghum. *Proceedings of the National Academy of Sciences of the United States of America*, 113: 8209–8209.

Chen, C., Mitchell, S.E., Elshire, R.J., Buckler, E.S., El-Kassaby, Y.A., 2013. Mining conifers' mega-genome using rapid and efficient multiplexed high-throughput genotyping-by-sequencing (GBS) SNP discovery platform. *Tree Genetics and Genomes*, 9: 1537–1544.

Chen, J., Liu, C., Shi, B., Chai, Y., Han, N., Zhu, M., Bian, H., 2017. Overexpression of *HvHGGT* Enhances Tocotrienol Levels and Antioxidant Activity in Barley. *Journal of Agricultural and Food Chemistry*, 7: 439.

Chen, M.-H., Bergman, C.J., 2015. Vitamin E homologs and γ-oryzanol levels in rice (*Oryza sativa L.*) during seed development. *Cereal Chemistry Journal*, CCHEM-07-15-0152-R.

Chiu, J., March, P.E., Lee, R., Tillett, D., 2004. Site-directed , Ligase-Independent Mutagenesis (SLIM): a single-tube methodology approaching 100 % efficiency in 4 h. *Nucleic Acids Research*, 32: 174-179.

Choong, C.G., McKay, A., 2014. Sustainability in the Malaysian palm oil industry. *Journal of Clean Production*, 85: 258–264.

Clough, S.J. and Bent, A.F., 1998. Floral dip: A simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana. The Plant Journal*, 16: 735-743.

Collakova, E., DellaPenna, D., 2001. Isolation and Functional Analysis of Homogentisate Phytyltransferase from *Synechocystis sp*. PCC 6803 and *Arabidopsis*. *Plant Physiology*, 127: 1113–1124.

Cordoba, E., Salmi, M., León, P., 2009. Unravelling the regulatory mechanisms that modulate the MEP pathway in higher plants. *Journal of Experimental Botany*, 60: 2933–43.

Corley, P., and Re, .H.V., 2007. The Products of the Oil Palm and Their Extraction, in: The Oil Palm. *Blackwell Science* Ltd, Oxford, UK, 445–466.

Corrêa, T.R., Motoike, S.Y., Coser, S.M., da Silveira, G., de Resende, M.D.V., Chia, G.S., 2015. Estimation of genetic parameters for *invitro* oil palm characteristics (*Elaeis guineensis Jacq*.) and selection of genotypes for cloning capacity and oil yield. *Industrial Crops and Production*, 77: 1033–1038.

Das, S., Powell, S.R., Wang, P., Divald, A., Nesaretnam, K., Tosaki, A., Cordis, G. A., Maulik, N., Das, D.K., 2005. Cardioprotection with palm tocotrienol: antioxidant activity of tocotrienol is linked with its ability to stabilize proteasomes. *American Journal of Physiology Heart Circulation and Physiology*, 289: 361-367.

Dellapenna D.A., 2005. A decade of progress in understanding vitamin E synthesis in plants *Journal of Plant Physiology*, 162: 729-737

Derrington, I.M., Butler, T.Z., Collins, M.D., Manrao, E., Pavlenok, M., Niederweis, M., Gundlach, J.H., 2010. Nanopore DNA sequencing with MspA. *Proceedings of the National Academy of Sciences of the United States of America*, 107: 16060-16065.

Doyle, J.J and Doyle, L.J. 1990. Isolation of plant DNA from fresh tissue. *Focus*, 12: 13-15.

Drive, B., Louis, S., 2006. Gateway-compatible vectors for plant functional genomics and proteomics. *The Plant Journal*, 45: 616–629.

Duran, C., Edwards, D., Batley, J., 2009. Molecular Marker Discovery and Genetic Map Visualisation, in: *Bioinformatics*. Springer New York, 165–189.

Ebrahimi, M., Nor, S., Abdullah, A., Aziz, M.A., Namasivayam, P., 2015. A novel CBF that regulates abiotic stress response and the ripening process in oil palm (*Elaeis guineensis*) fruits. *Tree Genetics & Genomes*, 11: 56

Eid, J., Fehr, A., Gray, J., Luong, K., Lyle, J., Otto, G., Peluso, P., Rank, D., Baybayan, P., Bettman, B., Bibillo, A., Bjornson, K., Chaudhuri, B., Christians, F., Cicero, R., Clark, S., Dalal, R., deWinter, A., Dixon, J., Foquet, M., Gaertner, A., Hardenbol, P., Heiner, C., Hester, K., Holden, D., Kearns, G., Kong, X., Kuse, R., Lacroix, Y., Lin, S., Lundquist, P., Ma, C., Marks, P., Maxham, M., Murphy, D., Park, I., Pham, T., Phillips, M., Roy, J., Sebra, R., Shen, G., Sorenson, J., Tomaney, A., Travers, K., Trulson, M., Vieceli, J., Wegener, J., Wu, D., Yang, A., Zaccarin, D., Zhao, P., Zhong, F., Korlach, J., Turner, S., 2009. Real-Time DNA Sequencing from Single Polymerase Molecules. *Science*, 80: 323.

Foster, J.T., Allan, G.J., Chan, A.P., Rabinowicz, P.D., Ravel, J., Jackson, P.J., Keim, P., 2010. Single nucleotide polymorphisms for assessing genetic diversity in castor bean (*Ricinus communis*). *BMC Plant Biology*, 10: 13.

Ghosh, S., Mandi, S. Sen, 2015. SNP in Chalcone Synthase gene is associated with variation of 6-gingerol content in contrasting landraces of *Zingiber officinale.Roscoe*. *Gene*, 566: 184-188.

Giraud, T., Refrégier, G., Le Gac, M., De Vienne, D.M., Hood, M.E., 2008. Speciation in fungi. *Fungal Genetic and Biology*, 45: 791-802.

Haller, G., Alvarado, D., Mccall, K., Mitra, R.D., Dobbs, M.B., Gurnett, C.A., 2016. Massively parallel single- nucleotide mutagenesis using reversibly terminated inosine. *Nature Methods*, 13: 923–925.

Han, M., May, Y., Ngan, A., Hock, C., Ali, M., 2004. Separation of Vitamin E (tocopherol, tocotrienol, and tocomonoenol) in Palm Oil. *Lipids*, 39: 1031–1035.

Hassanien, M.M.M., Abdel-Razek, A.G., Rudzińska, M., Siger, A., Ratusz, K., Przybylski, R., 2014. Phytochemical contents and oxidative stability of oils from non-traditional sources. *European Journal of Lipid Science and Technology*, 116: 1563–1571.

Hayashi, K., Hashimoto, N., Daigen, M., Ashikawa, I., 2004. Development of PCR-based SNP markers for rice blast resistance genes at the Piz locus. *Theoretical and Applied Genetics*, 108: 1212–1220.

Hayward, A.C., Tollenaere, R., Dalton-Morgan, J., Batley, J., 2015. Molecular Marker Applications in Plants. *Methods in Molecular Biology*, 1245: 13–27.

Heckman, K.L., Pease, L.R., 2007. Gene splicing and mutagenesis by PCR-driven overlap extension. *Nature Protocol*, 2: 924–932.

Horvath, G., Wessjohann, L., Bigirimana, J., Jansen, M., Guisez, Y., Caubergs, R., Horemans, N., 2006. Differential distribution of tocopherols and tocotrienols in photosynthetic and non-photosynthetic tissues. *Phytochemistry*, 67: 1185–1195.

Huang, X., Han, B., 2014. Natural Variations and Genome-Wide Association Studies in Crop Plants. *Annual Review of Plant Biology*, 65: 531–551.

Hunter, S.C., Cahoon, E.B., 2007. Enhancing vitamin E in oilseeds: unraveling tocopherol and tocotrienol biosynthesis. *Lipids*, 42: 97–108.

Huq, A., Akter, S., Nou, S., Kim, H.T., Jung, Y.J., Kang, K.K., 2016. Identification of functional SNPs in genes and their effects on plant phenotypes. *Journal of Plant Biotechnology*, 43: 1–11.

Husain, K., Centeno, B.A., Chen, D.-T., Hingorani, S.R., Sebti, S.M., Malafa, M.P., 2013. Vitamin E δ-tocotrienol prolongs survival in the LSL-KrasG12D/+; LSL-Trp53R172H/+ Pdx-1-Cre (KPC) transgenic mouse model of pancreatic cancer. *Cancer Prevention Research (Philadelphia)*, 6: 1074–1083.

Idrees, M., Irshad, M., 2015. Molecular Markers in Plants for Analysis of Genetic Diversity: A Review. *European Academic Research*, 2: 1513–1540.

Joshi, V., Joung, J.G., Fei, Z., Jander, G., 2010. Interdependence of threonine, methionine and isoleucine metabolism in plants: Accumulation and transcriptional regulation under abiotic stress. *Amino Acids*, 39: 933–947.

Juan, G., Liu, G., Shuangyan, C., Aly, A.A., 2009. Vitamin E Metabolic Modulation in Plants, in: Herbal Drugs: *Ethnomedicine to Modern Medicine*, Springer Berlin, Heidelberg, Germany. 333–352.

Kannappan, R., Gupta, S.C., Kim, J.H., Aggarwal, B.B., 2012. Tocotrienols fight cancer by targeting multiple cell signaling pathways. *Genes and Nutrition*, 7: 43-57.

Karimi, M., Inzé, D., Depicker, A., 2002. Gateway vectors for *Agrobacterium*-mediated plant transformation. *Trends in Plant Science*, 7: 193–195.

Karunanandaa, B., Qi, Q., Hao, M., Baszis, S.R., Jensen, P.K., Wong, Y.-H.H., Jiang, J., Venkatramesh, M., Gruys, K.J., Moshiri, F., Post-Beittenmiller, D., Weiss, J.D., Valentin, H.E., 2005. Metabolically engineered oilseed crops with enhanced seed tocopherol. *Metabolic Engineering*, 7: 384–400.

Kaur, J., Sharma, R., 2006. Directed Evolution: An Approach to Engineer Enzymes. *Critical Review in Biotechnology*, 26: 165–199.

Khanna, S., Patel, V., Rink, C., Roy, S., Sen, C., 2005. Delivery of orally supplemented α-tocotrienol to vital organs of rats and tocopherol-transport protein deficient mice. *Radical Biology and Medicine*, 39: 1310- 1319.

Kim, Y.H., Lee, Y.Y., Kim, Y.H., Choi, M.S., Jeong, K.H., Lee, S.K., Seo, M.J., Yun, H.T., Lee, C.K., Kim, W.H., Lee, S.C., Park, S.K., Park, H.M., 2011. Antioxidant Activity and Inhibition of Lipid Peroxidation in Germinating Seeds of Transgenic Soybean Expressing *OsHGGT*. *Journal of Agricultural and Food Chemistry*, 59: 584–591.

Kiryu, I., Nishioka, T., Yuasa, K., Kurita, J., Shimahara, Y., Ototake, M., Ikegami, N., Oseko, N., 2014. Rapid and Simple Detection Method of *Candidatus Xenohaliotis californiensis* Using Fecal PCR in Abalone *Haliotis discus discus* and *H. gigantea*. *Fish Pathology*, 49: 41–48.

Kong, S.L., Abdullah, S.N.A., Ho, C.L., Amiruddin, M.D., 2016. Molecular cloning, gene expression profiling and in silico sequence analysis of vitamin E biosynthetic genes from the oil palm. *Plant Gene*, 5: 100–108.

Koornneef, M., Meinke, D., Linne, C., West, R., 2010. The development of *Arabidopsis* as a model plant. *The Plant Journal*, 61: 909–921.

Krivanek, O.L., Chisholm, M.F., Nicolosi, V., Pennycook, T.J., Corbin, G.J., Dellby, N., Murfitt, M.F., Own, C.S., Szilagyi, Z.S., Oxley, M.P., Pantelides, S.T., Pennycook, S.J., 2010. Atom-by-atom structural and chemical analysis by annular dark-field electron microscopy. *Nature*, 464: 571–574.

Kumar, B., Abdel-ghani, A.H., Pace, J., Reyes-matamoros, J., Hochholdinger, F., Lübberstedt, T., 2014. Plant Science Association analysis of single nucleotide polymorphisms in candidate genes with root traits in maize (*Zea mays L.*) seedlings. *Plant Science*, 224: 9–19.

Kushairi, A, Tarmizi, A. H., Zamzuri, I., R, S.K., Ooi, S.E., Palm, M., Board, O., Institusi, N.P., Bangi, B.B., 2010. Production, Performance and Advances in Oil Palm Tissue Culture. *Internatinal Seminar Advance in Oil Palm Tissue Culture*, 1–23.

Lee, C.P., Taylor, N.L., Millar, A.H., 2013. Recent advances in the composition and heterogeneity of the *Arabidopsis* mitochondrial proteome. *Fronteirs in Plant Science*, 4: 4.

Leonelli, S., 2007. *Arabidopsis*, the botanical *Drosophila*: from mouse cress to model organism. *Endeavour*, 31: 34–38.

Liu, J., Huang, S., Sun, M., Liu, S., Liu, Y., Wang, W., Zhang, X., Wang, H., Hua, W., 2012. An improved allele-specific PCR primer design method for SNP marker analysis and its application. *Plant Methods*, 8: 34.

Luan, B., Peng, H., Polonsky, S., Rossnagel, S., Stolovitzky, G., Martyna, G., 2010. Base-By-Base Ratcheting of Single Stranded DNA through a Solid-State Nanopore. *Physical Review Letters*, 104: 238103.

Ma, Y., Coyne, C.J., Grusak, M.A., Mazourek, M., Cheng, P., Main, D., McGee, R.J., 2017. Genome-wide SNP identification, linkage map construction and QTL mapping for seed mineral concentrations and contents in pea (*Pisum sativum L.*). *BMC Plant Biology*, 17: 43.

Maarasyid, C., Muhamad, I.I., Supriyanto, E., 2014. Potential Source and Extraction of Vitamin E From Palm - Based Oils : A Review. *Journal Teknologi*, 4: 43–50.

May, C.Y., Nesaretnam, K., 2014. Highlight Article Research advancements in palm oil nutrition. *European Journal of Lipid Science and Technology*, 116: 1301–1315.

McCullum, E.O., Williams, B.A.R., Zhang, J., Chaput, J.C., 2010. Random mutagenesis by error-prone PCR. *Methods in Molecular Biology*, 634: 103–109.

Meinke, D.W., 1998. *Arabidopsis thaliana*: A Model Plant for Genome Analysis. *Science*, 80: 662–682.

Munusamy, U., Abdullah, S.N.A., Aziz, M.A., Khaza`Ai, H., 2015. Metabolic Engineering Of A-Tocotrienolthrough PGTS Mechanisms Andisoprenoid/Non-Mevalonate Pathways in perennial Crops. *Plant Cell Biotechnology and Molecular Biology*, 16: 119–129.

Nesaretnam, K., Meganathan, P., Veerasenan, S.D., Selvaduray, K.R., 2012. Tocotrienols and breast cancer: The evidence to date, in: *Genes and Nutrition*, 7: 3–9.

Paez-Garcia, A., Motes, C., Scheible, W.-R., Chen, R., Blancaflor, E., Monteros, M., 2015. Root Traits and Phenotyping Strategies for Plant Improvement. *Plants*, 4: 334–355.

Pavlov, M.Y., Watts, R.E., Tan, Z., Cornish, V.W., Ehrenberg, M., Forster, A.C., 2009. Slow peptide bond formation by proline and other N-alkylamino acids in translation. *Proceedings of the National Academy of Sciences*, 106: 50–54.

Peh, H.Y., Ho, W.E., Cheng, C., Chan, T.K., Seow, A.C.G., Lim, A.Y.H., Fong, C.W., Seng, K.Y., Ong, C.N., Wong, W.S.F., 2015. Vitamin E Isoform γ-Tocotrienol Downregulates House Dust Mite–Induced Asthma. *The Journal of Immunology*, 195: 2.

Pfaffl, M. W., Horgan, G. W. and Dempfle, L. 2002. Relative expression software tool (REST) for group- wise comparison and statistical analysis of relative expression results in real-time PCR. *Nucleic Acids Research*, 30: 36

Pootakham, W., Jomchai, N., Ruang-areerate, P., Shearman, J.R., Sonthirod, C., Sangsrakru, D., Tragoonrung, S., Tangphatsornruang, S., 2015. Genome-wide SNP discovery and identification of QTL associated with agronomic traits in oil palm using genotyping-by-sequencing (GBS). *Genomics*, 105: 288–295.

Prescott, A. and Martin, C., 1987. A rapid method for the quantitative assessment of levels of specific mRNAs in plants. *Plant Molecular Biology Report*, 4: 219-224.

Provart, N.J., Alonso, J., Assmann, S.M., Bergmann, D., Brady, S.M., Brkljacic, J., Browse, J., Chapple, C., Colot, V., Cutler, S., Dangl, J., Ehrhardt, D., Friesner, J.D., Frommer, W.B., Grotewold, E., Meyerowitz, E., Nemhauser, J., Nordborg, M., Pikaard, C., Shanklin, J., Somerville, C., Stitt, M., Torii, K.U., Waese, J., Wagner, D., Mccourt, P., 2016. 50 years of *Arabidopsis* research: Highlights and future directions. *New Phytologist*, 209: 921–944.

Quail, M., Smith, M.E., Coupland, P., Otto, T.D., Harris, S.R., Connor, T.R., Bertoni, A., Swerdlow, H.P., Gu, Y., Rothberg, J., Hinz, W., Rearick, T., Schultz, J., Mileski, W., Davey, M., Leamon, J., Johnson, K., Milgrew, M., Edwards, M., Eid, J., Fehr, A., Gray, J., Luong, K., Lyle, J., Otto, G., Peluso, P., Rank, D., Baybayan, P., Bettman, B., Bentley, D., Balasubramanian, S., Swerdlow, H., Smith, G., Milton, J., Brown, C., Hall, K., Evers, D., Barnes, C., Bignell, H., Kozarewa, I., Ning, Z., Quail, M., Sanders, M., Berriman, M., Turner, D., Quail, M., Otto, T., Gu, Y., Harris, S., Skelly, T., McQuillan, J., Swerdlow, H., Oyola, S., Syed, F., Grunenwald, H., Caruccio, N., Lam, H., Clark, M., Chen, R., Chen, R., Natsoulis, G., O'Huallachain, M., Dewey, F., Habegger, L., Carver, T., Harris, S., Berriman, M., Parkhill, J., McQuillan, J., Ponsting, N., Ning, Z., Otto, T., Sanders, M., Berriman, M., Newbold, C., Nakamura, K., Oshima, T., Morimoto, T., Ikeda, S., Yoshikawa, H., Shiwa, Y., Ishikawa, S., Linak, M., Hirai, A., Takahashi, H., Diep, B., Gill, S., Chang, R., Phan, T., Chen, J., Davidson, M., Lin, F., Lin, J., Carleton, H., Mongodin, E., Achidi, E., Gardner, M., Hall, N., Fung, E., White, O., Berriman, M., Hyman, R., Carlton, J., Pain, A., Nelson, K., Bowman, S., Choi, M., Scholl, U., Ji, W., Liu, T., Tikhonova, I., Zumbo, P., Nayir, A., Bakkaloglu, A., Ozen, S., Sanjad, S., Down, T., Rakyan, V., Turner, D., Flicek, P., Li, H., Kulesha, E., Graf, S., Johnson, N., Herrero, J., Tomazou, E., Giresi, P., Kim, J., McDaniell, R., Iyer, V., Lieb, J., Johnson, D., Mortazavi, A., Myers, R., Wold, B., Langridge, G., Phan, M., Turner, D., Perkins, T., Parts, L., Haase, J., Charles, I., Maskell, D., Peters, S., Dougan, G., Licatalosi, D., Mele, A., Fak, J., Ule, J., Kayikci, M., Chi, S., Clark, T., Schweitzer, A., Blume, J., Wang, X., Mamanova, L., Andrews, R., James, K., Sheridan, E., Ellis, P., Langford, C., Ost, T., Collins, J., Turner, D., Myllykangas, S., Buenrostro, J., Natsoulis, G., Bell, J., Ji, H., Shao, N., Hu, H., Yan, Z., Xu, Y., Hu, H., Menzel, C., Li, N., Chen, W., Khaitovich, P., Wang, Z., Gerstein, M., Snyder, M., Gnerre, S., Maccallum, I., Przybylski, D., Ribeiro, F., Burton, J., Walker, B., Sharpe, T., Hall, G., Shea, T., Sykes, S., Levin, J., Yassour, M., Adiconis, X., Nusbaum, C., Thompson, D., Friedman, N., Gnirke, A., Regev, A., Adey, A., Asan, Xun, X., Kitzman, J., Turner, E., Stackhouse, B., MacKenzie, A., Caruccio, N., Zhang, X., Flusberg, B., Webster, D., Lee, J., Travers, K., Olivares, E., Clark, T., Korlach, J., Turner, S., Holden, T., Lindsay, J., Corton, C., Quail, M., Cockfield, J., Pathak, S., Batra, R., Parkhill, J., Bentley, S., Edgeworth, J., Li, H., Durbin, R., Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., Durbin, R., Angiuoli, S., Salzberg, S., 2012. A tale of three next generation

sequencing platforms: comparison of Ion torrent, pacific biosciences and illumina MiSeq sequencers. *BMC Genomics*, 13: 341.

Qureshi, A.A., Sami, S.A., Salser, W.A., Khan, F.A., 2002. Dose-dependent suppression of serum cholesterol by tocotrienol-rich fraction (TRF25) of rice bran in hypercholesterolemic humans. *Atherosclerosis*, 161: 199–207.

Rajanaidu, N., kushairi H., 2004. Ps8: high vitamin e breeding population. MPOB Information Series, *Science Technology*, 229.

Rajanaidu, N.; Johari, O.; Ahmad, S. F. Veriappan, A. 2008. Proccedings of the 3rd Seminar on performance of MPOB PS1 and PS2 materials and elite ge: MPOB Head Office, Bandar Baru Bangi, Selangor. 91-116

Ramli, A., 2015. Strengthening Agricultural Sector Superior Commodities -Based Against the Economic Growth in South Sulawesi, Indonesia. *International Journal of Advanced Research*, 3: 753–760.

Reid, J.G., Carroll, A., Veeraraghavan, N., Dahdouli, M., Sundquist, A., English, A., Bainbridge, M., White, S., Salerno, W., Buhay, C., Yu, F., Muzny, D., Daly, R., Duyk, G., Gibbs, R.A., Boerwinkle, E., 2014. Launching genomics into the cloud: deployment of Mercury, a next generation sequence analysis pipeline. *BMC Bioinformatics*, 15: 30.

Rhoads, A., Au, K.F., 2015. PacBio Sequencing and Its Applications. *Genomics, Proteomics and Bioinformatics*, 13, 278–289.

Riju, A., Arunachalam, V., 2009. Interspecific differences in single nucleotide polymorphisms (SNPs) and indels in expressed sequence tag libraries of oil palm *Elaeis guineensis* and *E. oleifera*. *Nature Precedings*, 3593: 1.

Rival Alain and Parveez Ghulam, 2014. Biotechnology of Fruit and Nut Crops - Richard E Litz (Ed.), CABI Publishing, Massachusset USA, 113–136.

Roslinda, Sajari., NH Abd Razak., Farida Yusof., S.A., 2014. Improved Efficiency of Tocotrienol Extraction from Fresh and Processed Latex. *Journal of Rubber Research*, 17: 245–260.

Schadt, E.E., Turner, S., Kasarskis, A., 2010. A window into third-generation sequencing. *Human Molecular Genetics*, 19: 227–240.

Sekine, D., Murata, K., Kimura, T., Nakagawa, K., Miyazawa, T., 2016. Identification of a Genetic Factor Required for High γ-Isoform Concentration in Rice Vitamin E. *Journal of Agricultural and Food Chemistry*, 64: 9368–9373.

Semagn, K., Babu, R., Hearne, S., Olsen, M., 2014. Single nucleotide polymorphism genotyping using Kompetitive Allele Specific PCR (KASP): Overview of the technology and its application in crop improvement. *Molecular Breeding*, 33: 1–14.

Sen, C.K., Khanna, S., Roy, S., 2006. Tocotrienols: Vitamin E beyond tocopherols. *Life Science*, 78: 2088–2098.

Shahidi, F., De Camargo, A., 2016. Tocopherols and Tocotrienols in Common and Emerging Dietary Sources: Occurrence, Applications, and Health Benefits. *Intertional Journal of Molecular Sciences*, 17: 1745

Shahidi, F., Zhong, Y., Song, C., Ahn, C.B., Shin, T.S., Cha, Y.J., Shahidi, F., Jeon, Y.J., 2010. Lipid oxidation and improving the oxidative stability. *Chemical Society Review*, 39: 4067.

Shapiro, E., Biezuner, T., Linnarsson, S., 2013. Single-cell sequencing-based technologies will revolutionize whole-organism science. *Nature Review Genetics*, 14: 618–630.

Sheppard, A.J., Pennington, J.A.T., Weihrauch, J.L., 1993. Analysis and distribution of vitamin E in vegetable oils and foods. *Vitamin E in Health and Disease*, 9: 31.

Shi, Z., Liu, S., Noe, J., Arelli, P., Meksem, K., Li, Z., 2015. SNP identification and marker assay development for high-throughput selection of soybean cyst nematode resistance. *BMC Genomics*, 16: 314.

Singh, R., Ong-Abdullah, M., Low, E.-T.L., Manaf, M.A.A., Rosli, R., Nookiah, R., Ooi, L.C.-L., Ooi, S.-E., Chan, K.-L., Halim, M.A., Azizi, N., Nagappan, J., Bacher, B., Lakey, N., Smith, S.W., He, D., Hogan, M., Budiman, M.A., Lee, E.K., DeSalle, R., Kudrna, D., Goicoechea, J.L., Wing, R.A., Wilson, R.K., Fulton, R.S., Ordway, J.M., Martienssen, R.A., Sambanthamurthi, R., 2013. Oil palm genome sequence reveals divergence of interfertile species in Old and New worlds. *Nature*, 500: 335–339.

Sylvester, P.W and Theriault, A., 2003. Role of Tocotrienols in the Prevention of Cardiovascular Disease and Breast Cancer. *Current Topics in Nutraceutical Research*, 1: 121-136.

Stacey, G.S. and M.G., 2013. Metabolic engineering of plants for increased homogentisate and tocochromanol production. *Patent US 9624500 B2*.

Suzuki, Y., Tsuchiya, M., Wassall, S., 1993. Structural and dynamic membrane properties of. Alpha.-tocopherol and. Alpha-tocotrienol: implication to the molecular mechanism of their antioxidant potency. *Biochemistry*, 32: 10692–10699.

Tavva, V.S., Kim, Y.H., Kagan, I.A., Dinkins, R.D., Kim, K.H., Collins, G.B., 2007. Increased α-tocopherol content in soybean seed overexpressing the *Perilla frutescens* γ-tocopherol methyltransferase gene. *Plant Cell Reports*, 26, 61–70.

Teoh C.H., 2002. The palm oil industry in malaysia from seed to frying pan prepared for the palm oil industry in malaysia: from seed to frying pan. *Plantation Agriculture*, WWF Malaysia, 49, Jalan SS 23/15. Taman SEA, 47400 Petaling Jaya, Selangor, Malaysia

Theriault, A., Chao, J.-T., Wang, Q., Gapor, A., Adeli, K., 1999. Tocotrienol: A review of its therapeutic potential. *Clinical Biochemistry*, 32: 309–319.

Urban, A., 1997. A rapid and efficient method for site-directed mutagenesis using one-step overlap extension PCR. *Nucleic Acids Research*, 25: 2227–2228.

Valentin, H.E., Qi, Q., 2005. Biotechnological production and application of vitamin E: Current state and prospects. *Applied Microbiology and Biotechnology*, 68: 436–444.

Vangelatos, I., Vlachakis, D., Sophianopoulou, V., Diallinas, G, 2009. Modelling and mutational evidence identify the substrate binding site and functional elements in APC amino acid transporters. *Molecular Membrane Biology*, 26: 356–370.

Verhage, L., Angenent, G.C., Immink, R.G.H., 2014. Research on floral timing by ambient temperature comes into blossom. *Trends in Plant Science*, 19: 583–591.

Wahid, M.B., Abdullah, S.N.A., Henson, I.E., 2005. Oil Palm-Achievements and Potential. *Plant Production Science*, 8: 288–297.

Wang, C., Husain, K., Zhang, A., Centeno, B.A., Chen, D., Tong, Z., Sebti, S.M., Malafa, M.P., 2015. ScienceDirect EGR-1 / Bax pathway plays a role in vitamin E δ - tocotrienol-induced apoptosis in pancreatic cancer cells. *Journal of Nutritional Biochemistry*, 26: 797–807.

Wang, J., Sun, N., Deng, T., Zhang, L., Zuo, K., 2014. Genome-wide cloning, identification, classification and functional analysis of cotton heat shock transcription factors in cotton (*Gossypium hirsutum*). *BMC Genomics*, 15: 961.

Wangkumhang, P., Chaichoompu, K., Ngamphiw, C., Ruangrit, U., Chanprasert, J., Assawamakin, A., Tongsima, S., 2007. WASP: a Web-based Allele-Specific PCR assay-designing tool for detecting SNPs and mutations. *BMC Genomics*, 8: 275.

Weigel, D., and Glazebrook, J. 2002. *Arabidopsis*. *Cold Spring Harbor Laboratory Press*. Cold Spring Harbor: Retrieved from http://tocs.ulb.tudarmstadt.de/108129926.pdf

Whiteford, N., Skelly, T., Curtis, C., Ritchie, M.E., Löhr, A., Zaranek, A.W., Abnizova, I., Brown, C., 2009. Swift: Primary data analysis for the Illumina Solexa sequencing platform. *Bioinformatics*, 25: 2194–2199.

Winifred, M.C., Thelma, S.V., Edward, H.W., Beverly, D.W., 1983. Vitamin E content of feedstuffs determined by high performance liquid chromatographic fluorescence. *Journal of Agricultural and Food Chemistry*, 31: 1330-1333.

Woittiez, L. S., van Wijk, M. T., Slingerland, M., van Noordwijk, M., Giller, K.E., 2017. Yield gaps in oil palm: A quantitative review of contributing factors. *European Journal of Agronomy*, 83: 57–77.

Wong, R.S.Y., Radhakrishnan, A.K., 2012. Tocotrienol research: past into present. *Nutrition Reviews*, 70: 483–90.

Yang, W., Cahoon, R.E., Hunter, S.C., Zhang, C., Han, J., Borgschulte, T., Cahoon, E.B., 2011. Vitamin E biosynthesis : functional characterization of the monocot *homogentisate geranylgeranyl transferase*. *The Plant Journal*, 65: 206–217.

Zaki, N.M., Singh, R., Rosli, R., Ismail, I., 2012. Elaeis oleifera Genomic-SSR Markers: Exploitation in Oil Palm Germplasm Diversity and Cross-Amplification in Arecaceae. *International Journal of Molecular Science*, 13: 4069–4088.

Zeng, F., Zhang, Y., Zhang, Z., Malik, A.A., Lin, Y., Zeng, F., 2017. Multiple-site fragment deletion, insertion and substitution mutagenesis by modified overlap extension PCR 2818. *Biotechnology & Biotechnological Equipment*, 31: 339–348

Zhang, C., Cahoon, R.E., Hunter, S.C., Chen, M., Han, J., Cahoon, E.B., 2013. Genetic and biochemical basis for alternative routes of tocotrienol biosynthesis for enhanced vitamin e antioxidant production. *The Plant Journal*, 73: 628–639.

Zhang, G.Y., Liu, R.-R., Xu, G., Zhang, P., Li, Y., Tang, K. X., Liang, G. H., Liu, Q. Q., 2013. Increased α-tocotrienol content in seeds of transgenic rice overexpressing *Arabidopsis* γ-tocopherol methyltransferase. *Transgenic Research*, 22, 89–99.