



UNIVERSITI PUTRA MALAYSIA

***MOLECULAR-PHYSIOLOGICAL RESPONSE OF OIL PALM SEEDLINGS
TO DROUGHT STRESS AND FUNCTIONAL CHARACTERIZATION OF
EgDREB1 IN TRANSGENIC TOMATO***

AZZREENA BINTI MOHAMAD AZZEME

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By

AZZREENA BINTI MOHAMAD AZZEME

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

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This thesis is dedicated to all loves, especially to my parents, husband, siblings and all of my family members who have given great support, motivation and prayers since the beginning of my journey to complete this thesis.



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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June 2015

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Drought is an insidious natural hazard that imposes serious challenges to agricultural activities in the world. It causes losses of major food crops, interfering food chains and losses of world economic worth of million dollars every year. In Malaysia, climate change such as *El Nino* has become a major problem that gives negative impact to environment. *El Nino* has an ability to bring worst drought phenomena. Apart from that, even though Malaysia receives an average rainfall of 2000 mm annually, there are certain areas still have low amount of rainfall such as Kedah and Perlis. The low rainfall period can prolong up to two to three months. Thus, it may give negative impacts to oil palm (*Elaeis guineensis* Jacq.) plantation, because adequate water is essential for healthy growth and maximum performance of oil palm seedlings. Therefore, this study was conducted to determine physiological and molecular changes of oil palm seedlings in response to different severity of drought stress. To achieve the objective, a study that links the symptoms under different drought severity with physiological and molecular responses was carried out. Five durations of drought treatments (7, 14, 21, 28, 35 days of water withholding; DWW) were given to 5-month-old seedlings. The necrosis, chlorosis and burned symptoms started to appear in seedling leaves at 21 DWW (severe drought). However, the leaf physiological data showed photosynthetic rate (A), stomatal conductance (gs) and transpiration rate (E) started to decrease earlier as at 7 DWW (mild drought) before any stress morphological symptoms in leaves were established. Drought-responsive element binding 1 (DREB1) belongs to AP2 superfamily of plant specific transcription factor (TF). Early accumulation of the oil palm *EgDREB1* transcript (>1-fold) in roots might be associated with signaling pathway; while the significant up-regulation of *EgDREB1* in leaves under severe drought corresponded to the high *peroxidase* (POD) antioxidant gene expression in roots. *Catalase* (CAT), *superoxide dismutase* (SOD), *ascorbate peroxidase* (APX) and *glutathione reductase* (GR) antioxidant genes which were highly up-regulated under moderate drought in leaves may be involved in scavenging reactive oxygen species (ROS) and ensuring water balance in this tissue. The *ethylene responsive binding protein* (EREBP), *late embryogenesis abundant* (LEA), *dehydrin* (DHN), *cold-induced* (CI), *heat shock*

protein 70 (HSP70) and *metallothionein type 2 (MET2)* were differentially up-regulated in the leaves, while in roots only the LEA protein genes (*LEA* and *DHN*) were up-regulated. The diminishing total chlorophyll (chl) content and the ratio of chl_a to chl_b (chl_a:chl_b) were significantly observed ($P < 0.05$). The significant reduction of chl_a was closely related to the deficiency of photosystem II (PSII). The proline content increased gradually in both vegetative tissues, while the total soluble protein content was affected by increasing drought severity. The activity of the antioxidant enzyme, catalase (CAT; EC 1.11.1.6) was the highest in the root under severe drought stress, while guaiacol peroxidase (POD; EC 1.11.1.7) activity was shown to be the highest in the leaves under mild drought stress. The full amino acid sequence of the EgDREB1 was more closely related to the dicot NtDREB2. The subcellular localization, *in vivo* and *in vitro* DNA-protein binding assays further confirmed the function of EgDREB1 protein as a transcription factor (TF). Functional analysis was carried out in tomato by over-expressing *EgDREB1*, driven by a constitutive double cauliflower mosaic virus 35S promoter. The *in vitro* T₀ transgenic plants showed slower growth and dwarf phenotype under controlled conditions (24°C), and they produced parthenocarpic fruits and fruits with reduced seed numbers when grown in the transgenic greenhouse at ambient temperature (28-30°C) with direct sunlight even though they recovered from dwarfism symptom. Expression of *EgDREB1* was high in all transgenic fruits, but not detected in the leaves and roots. The expression of ethylene-responsive genes (*LeACS*, *LeACO* and *LeAP2*), jasmonate-responsive genes (*LeAOS* and *LeAOC*), auxin-responsive genes (*LeARF8* and *LeAux/IAA*), cytokinin-responsive genes (*LeSICKXI* and *LeSIPT1*), GA-responsive gene (*LeGA2ox2* and *LeGA2ox4*) and ABA-responsive gene (*LeAAO*) was regulated in a different manner between the seedless and low seed number phenotypes. This suggests the complex interplay between the different phytohormones in contributing to the abnormal fruit phenotype. *EgDREB1* transgene and endogenous SRGs like *LePOD*, *LeAPX*, *LeGP*, *LeCAT*, *LeHSP70*, *LeLEA*, *LeMET2*, *LePCS*, *LeSOD*, *LeGR*, *LeAAO* and *LeECD* were up-regulated in all seedlings of T₁ transgenic progeny under polyethylene glycol (PEG) treatment and cold stress (4°C). Hence, based on these findings, EgDREB1 might be involved in fruit and seed development, leaves formation, internodes elongation and adaptation to drought and cold stress.

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

**TINDAK BALAS MOLEKUL-FISIOLOGI ANAK BENIH KELAPA SAWIT
TERHADAP TEKANAN KEMARAU DAN PENCIRIAN BERFUNGSI
EgDREB1 DALAM TOMATO TRANSGENIK**

Oleh

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Kemarau adalah bencana alam berbahaya yang memberikan cabaran serius kepada aktiviti pertanian di dunia. Ia menyebabkan kehilangan tanaman makanan utama, mengganggu rantai makanan dan kerugian ekonomi dunia sebanyak jutaan dolar setiap tahun. Di Malaysia, perubahan iklim seperti *El Nino* telah menjadi masalah utama yang memberi kesan negatif kepada alam sekitar. *El Nino* mempunyai keupayaan untuk membawa fenomena kemarau paling teruk. Selain itu, walaupun Malaysia menerima purata hujan 2000 mm setahun, terdapat kawasan tertentu masih menerima jumlah hujan sukatan terendah seperti Kedah dan Perlis. Tempoh kadar hujan yang rendah boleh berpanjangan sehingga dua hingga tiga bulan. Oleh itu, ia boleh memberi kesan negatif kepada penanaman kelapa sawit (*Elaeis guineensis* Jacq.), kerana air yang mencukupi adalah penting untuk pertumbuhan yang sihat dan prestasi maksimum benih kelapa sawit. Dengan itu, kajian ini dijalankan untuk menentukan perubahan fisiologi dan molekul anak benih kelapa sawit sebagai tindak balas terhadap tahap tekanan kemarau yang berbeza. Untuk mencapai objektif ini, satu kajian yang menghubungkan gejala di bawah tahap kemarau yang berbeza dengan tindak balas fisiologi dan molekul telah dijalankan. Lima tempoh rawatan kemarau (7, 14, 21, 28, 35 tanpa air; DWW) telah diberikan kepada anak benih berusia 5 bulan. Gejala nekrosis, klorosis dan terbakar mula kelihatan di dalam daun anak benih pada 21 DWW (kemarau teruk). Walau bagaimanapun, data fisiologi daun menunjukkan kadar fotosintesis (A), kealiran stomata (gs) dan kadar transpirasi (E) mula berkurangan lebih awal pada 7 DWW (kemarau awal) sebelum gejala tekanan morfologi dalam daun kelihatan. "Drought-responsive element binding 1" (DREB1) tergolong dalam faktor transkripsi (TF) tumbuhan superfamili AP2. Pengumpulan awal transkrip *EgDREB1* kelapa sawit (>1 kali ganda) di dalam akar mungkin dikaitkan dengan tapak jalan pengisyaratan; manakala naikkawal *EgDREB1* yang signifikan di dalam daun pada kemarau teruk adalah sepadan dengan ekspresi gen antioksidan *peroxidase* (POD) yang tinggi di dalam akar. Gen antioksida *katalase* (CAT), *superoxide dismutase* (SOD), *askorbat peroxidase* (APX) dan *glutathion reductase* (GR) yang dinaikkawal pada kadar yang tinggi di dalam daun di peringkat kemarau sederhana mungkin terlibat dalam memerangkap spesies oksigen reaktif (ROS) dan untuk memastikan keseimbangan air di dalam tisu ini.

*Protein pengikat responsif etilena (EREBP), “late embryogenesis abundant” (LEA), “dehydrin” (DHN), “cold-induced” (CI), “heat shock protein 70” (HSP70) dan “metallothionein type 2” (MET2) telah dikawalnaik secara berbeza di dalam daun, manakala di dalam akar hanya gen protein LEA (LEA dan DHN) telah dikawalnaik. Pengurangan jumlah kandungan klorofil (chl) dan nisbah chl_a dan chl_b (chl_a: chl_b) diperhatikan dengan ketara ($P < 0.05$). Pengurangan ketara chl_a adalah berkait rapat dengan defisiensi photosystem II (PSII). Kandungan prolin telah meningkat secara beransur di dalam kedua-dua tisu vegetatif, manakala jumlah kandungan protein larut telah terjejas dengan peningkatan tahap kemarau. Aktiviti enzim antioksidan, katalase (CAT; EC 1.11.1.6) adalah paling tinggi di dalam akar pada peringkat kemarau yang teruk, manakala aktiviti guaicol peroxidase (POD; EC 1.11.1.7) berada pada kadar tertinggi di dalam daun pada peringkat awal tekanan. Urutan lengkap asid amino EgDREB1 lebih berkait rapat dengan NtDREB2 dikot. Penyetempatan subsekt, asai pengikat DNA-protein *in vivo* dan *in vitro* mengesahkan lagi fungsi protein EgDREB1 sebagai faktor transkripsi (TF). Analisis kefungsiannya telah dilakukan di dalam tomato melalui pengekspresan melampau *EgDREB1*, didorong oleh dua juzukan promoter virus cauliflower mosaic 35S. Tumbuhan transgenik T₀ *in vitro* menunjukkan pertumbuhan yang lebih perlahan dan fenotip kerdil di bawah keadaan terkawal (24°C), dan menghasilkan buah ‘parthenocarpic’ dan buah kekurangan bilangan biji apabila ia ditanam di rumah hijau transgenik dalam suhu ambien dan cahaya matahari langsung walaupun mereka pulih daripada gejala kerdil. Ekspresi *EgDREB1* telah dinaikkawal di dalam semua buah transgenik, tetapi tidak dikesan di dalam daun dan akar. Ekspresi gen responsif etilena (*LeACS*, *LeACO* dan *LeAP2*), gen responsif jasmonate (*LeAOS* dan *LeAOC*), gen responsif auksin (*LeARF8* dan *LeAux/IAA*), gen responsif cytokinin (*LeSICKXI* dan *LeSIIPT1*), gen responsif GA (*LeGA2ox2* dan *LeGA2ox4*) dan gen responsif ABA (*LeAAO*) telah dikawal di dalam cara yang berbeza antara buah tanpa biji dan buah kekurangan bilangan biji. Ini menunjukkan interaksi kompleks antara fitohormon yang berbeza dalam menyumbang kepada fenotip buah tidak normal. Transgen *EgDREB1* dan SRGs endogen seperti *LePOD*, *LeAPX*, *LeGP*, *LeCAT*, *LeHSP70*, *LeLEA*, *LeMET2*, *LePCS*, *LeSOD*, *LeGR*, *LeAAO* dan *LeECD* telah dinaikkawal di dalam semua progeni anak benih transgenik T₁ di bawah rawatan polyethylene glycol (PEG) dan tekanan sejuk (4°C). Maka, berdasarkan daripada penemuan-penemuan ini, *EgDREB1* berkemungkinan terlibat di dalam pengembangan buah, pembentukan daun, pemanjangan internod dan penyesuaian terhadap tekanan kemarau dan sejuk.*

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I certify that a Thesis Examination Committee has met on 17 June 2015 to conduct the final examination of Azzreena binti Mohamad Azzeme on her thesis entitled "Molecular-Physiological Response of Oil Palm Seedlings to Drought Stress and Functional Characterization of *EgDREB1* in Transgenic Tomato" 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.

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LIST OF ABBREVIATIONS

| | | |
|-----------------------|---|---|
| A | - | CO ₂ assimilation |
| ABA | - | Absciscic acid |
| ABRE | - | ABA-responsive element (ABRE) |
| AD | - | Activation domain |
| ADC | - | Arginine decarboxylase |
| AFB | - | Auxin F-box |
| AHK | - | Receptor histidine kinase |
| AHP | - | Histidine phospho-transfer protein |
| APX | - | Ascorbate peroxidase |
| ARE | - | Auxin responsive element |
| ARFs | - | Auxin response factors |
| AUX/IAA | - | Auxin/Indole-3-Acetic Acid |
| <i>A. tumefaciens</i> | - | <i>Agrobacterium tumefaciens</i> |
| AP2/ERF | - | APETALA 2/ethylene-responsive factor |
| AREB | - | ABA-responsive element binding |
| BADH | - | Betaine aldehyde dehydrogenase |
| BAP | - | Benzylaminopurine |
| bHLH | - | Basic-helix-loop-helix |
| BLAST | - | Basic Local Alignment Search Tool |
| bp | - | Base pair |
| BSA | - | Bovine serum albumin |
| bZIP | - | Basic leucine zipper containing domain proteins |
| CaMV 35S | - | Cauliflower Mosaic Virus 35S |
| CAT | - | Catalase |
| CaCO ₃ | - | Calcium carbonate |
| Ca ²⁺ | - | Calcium |
| CBF1 | - | C-repeat binding factor 1 |
| Chl | - | Chlorophyll |
| Chl _a | - | Chlorophyll a |
| Chl _b | - | Chlorophyll b |
| CK | - | Cytokinin |
| CI | - | Cold-induced |
| CO ₂ | - | Carbon dioxide |
| CPPU | - | cytokinin <i>N</i> -(2-chloro-pyridin-4-yl) - <i>N</i> '-phenylurea |
| CRD | - | Completely randomized design |
| CRT | - | C-repeat |
| CTAB | - | Cetyltrimethyl ammonium bromide |
| DEPC | - | Diethylpyrocarbonate |
| DHAR | - | Dehydroascorbate reductase |
| DHN | - | Dehydrin |
| DMSO | - | Dimethyl sulfoxide |
| DNA | - | Deoxyribonucleic acid |
| dNTPs | - | Deoxynucleotide |
| DREB | - | Drought-responsive element binding |
| DRE/CRT | - | Dehydration-responsive element/C-repeat |

| | |
|-------------------------------|---|
| DWW | - Days of water withholding |
| E | - Transpiration rate |
| EA1332 | - Unknown protein (rice) |
| EABF | - ABA-responsive binding factor |
| EDTA | - Ethylenediaminetetraacetic acid |
| EIN | - Ethylene insensitive 2 |
| EL | - Electrolytic leakage |
| EMSA | - Electrophoretic Mobility Shift Assay |
| EREBP | - Ethylene-responsive binding protein |
| ERF | - Ethylene responsive factor |
| EST | - Expressed sequence tag |
| ETR | - Ethylene receptor |
| FFB | - Fresh fruit bunch |
| GA | - Gibberellic acid |
| GAPDH | - Glyceraldehydes-3-phospahte dehydrogenase |
| GB | - Glycine betaine |
| GP | - Glutathione peroxidase |
| GR | - Glutathione reductase |
| gs | - Stomatal conductance |
| HDL | - High-density lipoprotein |
| HSPs | - Heat shock proteins |
| HSP70 | - Heat shock protein 70 |
| H ₂ O ₂ | - Hydrogen peroxide |
| IAA | - Indole-3-acetic acid |
| JA | - Jasmonate |
| Jacq. | - Jacquín |
| JIP | - Jasmonate-induced protein |
| KIN | - Kinetin |
| LDL | - Low-density lipoprotein |
| LEA | - Late embryogenesis abundant |
| LiCl | - Lithium chloride |
| LP | - Lipid peroxidation |
| LRR-RLKs | - Leucine-rich repeat receptor like kinase |
| LTRE | - Low temperature-responsive element |
| MAPKs | - Mitogen-activated protein kinases |
| MARDI | - Malaysian Agricultural Research and Development Institute |
| MET2 | - Metallothionein type-2 |
| MDHAR | - Monodehydroascorbate reductase |
| MgCl ₂ | - Magnesium chloride |
| MIC | - Minimal inhibitory concentration |
| MS | - Murashige and Skoog |
| MT1 | - MARDI tomato-1 |
| MT11 | - MARDI tomato-11 |
| MYC | - Myelocytomatosis |

| | |
|------------------------------|--|
| N | - Nitrogen |
| NAA | - 1-naphthaleneacetic acid |
| NAC | - NAM/ATAF1/CUC2 |
| NACRS | - NAC recognition sequence |
| NaCl | - Sodium chloride |
| NGO | - Non-government organizations |
| NLS | - Nuclear localization signals |
| NO | - Nitric oxide |
| NO ₃ ⁻ | - Nitrate |
| NO ₂ ⁻ | - Nitrite |
| NR | - Nitrate reductase |
| OD | - Optical density |
| ORF | - Open reading frame |
| PCD | - Programmed cell death |
| PCI | - Phenol: chloroform: isoamyl alcohol |
| PCR | - Polymerase Chain Reaction |
| <i>PD569</i> | - Manganese superoxide dismutase |
| PEG | - Polyethylene glycol |
| pI | - Isoelectric point |
| PSII | - Photosystem II |
| P5CS | - Δ^1 -pyrroline-5-carboxylate synthetase |
| qPCR | - Quantitative real-time PCR |
| RNA | - Ribonucleic acid |
| RNase | - Ribonuclease |
| RPKs | - Receptor protein kinases |
| RMK8 | - Eight Malaysia Plan |
| ROS | - Reactive oxygen species |
| RT-PCR | - Reverse transcription-PCR |
| RWC | - Relative water content |
| SA | - Salicylic acid |
| SDS | - Sodium dodecyl sulfate |
| SOD | - Superoxide dismutase |
| SPDS | - Spermidine synthase |
| SPS | - Sucrose-phosphate synthase |
| SRGs | - Stress-responsive genes |
| SURE | - Sugar-responsive cis-element |
| TAE | - Tris-acetate-EDTA |
| TBE | - Tris-borate-EDTA |
| TC | - Total chlorophyll |
| TDZ | - Thidiazuron |

| | | |
|----------------|---|-----------------------------------|
| T-DNA | - | Transferred-DNA |
| TE | - | Tris-EDTA |
| TFs | - | Transcription factors |
| TIRI | - | Transport inhibitor response 1 |
| T _m | - | melting temperature |
| Tris | - | Tris [hydroxymethyl] aminomethane |
| Tris-HCl | - | Tris-hydrochloride |
| U | - | Unit |
| UKM | - | Universiti Kebangsaan Malaysia |
| UTR | - | Untranslated region |
| WT | - | Wild type |
| WUE | - | Water use efficiency |
| μl | - | Microliter |
| μM | - | Micromolar |

CHAPTER 1

INTRODUCTION

Oil palm is an important oil crop commercially grown in Malaysia. Cultivation of the high yielding tenera hybrid (Dura X Pisifera) together with a strong infrastructure and technical know-how have led Malaysia to its present status as the second largest producer of palm oil after Indonesia (Gan and Li, 2014). There is a great demand for palm oil in the food sector mainly for producing cooking oil, margarines and shortenings and in the non-food sector as raw materials such as in producing detergents, cosmetics and biodiesel (Latip et al., 2013; Rashid et al., 2014; Siwayanan et al., 2014). Today, about 5.39 million hectares of land in Malaysia are being used for oil palm cultivation. (Malaysian Palm Oil Board, 2014). However, there are limited areas for further expansion, and available areas gazetted for agricultural activities in Malaysia may also be required for rubber plantations and for enhancing self-sufficiency in food production. Apart from that, certain oil palm plantations have been cleared for the development of new townships and industrial area as Malaysia is moving towards achieving a developed country status by the year 2020. The search and opening of new plantations in other countries with less suitable climate for oil palm cultivation by Malaysian companies may be catastrophic due to abiotic stress faced by the trees. Abiotic stress can cause the young palm seedlings become stunted and even result in plant death due to the high injury index in the plant tissues when they are planted in the field (Cao et al, 2011).

Abiotic stress is an adverse force or influence that tends to inhibit the biological system from functioning optimally in a normal plant (Mahajan and Tuteja, 2005). As a sessile living organism on the earth, plants are certainly affected by abiotic stresses like drought, flood, salinity, cold, extreme temperature and exposure to heavy metal ions. Extreme climate changes like *El Nino*, *La Nina* and global warming are major phenomena that can lead to major abiotic stress in plants. Abiotic stresses must be seriously addressed as they can lead to disastrous effects to agriculture and plantation industries due to crop loss and major drop in productivity. It was estimated that hundreds of million dollars are lost every year due to the effects of abiotic stresses on crop production (Schowalter, 2011).

Studies on the mechanisms of abiotic stress adaptation and response have been explored extensively. However, most of them have been intensively investigated in a model plant, the *Arabidopsis thaliana* (Jones, 2009). There has been no in depth studies carried out on the oil palm on the effect of abiotic stress on its growth and development. Primary perception of extreme condition from their surroundings leads to biochemical and physiological alterations in plants and transcriptional activation of stress-responsive genes (SRGs) as a recovery and adaptation system. It results in transcriptional activation of genes involved in production of osmo-protectants such as proline, glycinebetaine and mannitol and antioxidant enzymes and metabolites. Genes encoding products involved in protein turnover especially proteases

stress-signaling pathway like mitogen activated protein kinase and transcriptional regulation particularly transcription factors (Cabello et al., 2014; Danquah et al., 2014) are also transcriptionally activated.

Transcriptional regulation of the expression of SRGs is a critical part of the plant response to a range of abiotic stresses. The initial step when the SRGs are selected for expression during stress conditions and also during modulation of the transcription of the SRGs are controlled by transcription factors (TFs) (Vaahtera and Brosche, 2011; Prasch and Sonnewald, 2015). TFs are *trans*-acting proteins responsible for regulating expression of downstream genes. They act by binding to *cis*-acting elements in the promoters of the target genes and therefore they can activate or suppress the transcription of the target genes (Mizoi et al., 2011). Dehydration-responsive element binding (DREB) is a transcription factor commonly involved in regulating SRGs expression. DREB interacts with dehydration response element (DRE). The DREB family of transcription factors is involved in conferring drought, salt and cold tolerance in plants. Their protein sequences contain a highly conserved AP2/ERF domain of approximately 58 to 70 amino acids (Li et al., 2013; Zhang et al., 2014). Apart from that, the different functions of DREB family members such as DREB1 and DREB2 in different signaling pathways of abiotic stress remain controversial and not fully understood (Yoshida et al., 2014). DREB1A transcription factor is believed to be involved in modulation of cold stress response, while DREB2A is responsible in modulation of drought stress response (Nakashima et al., 2014). The gene functional study via ectopic expression of *DREB* in transgenic plants shows different phenotypic changes besides inducing abiotic stress tolerance. The phenotypic changes include growth retardation of transgenic plants and delayed flowering time. The changes are believed to be due to interference of gibberellic acid (GA) biosynthesis and metabolism (Agarwal, et al., 2006; Akhtar et al., 2012). However, different phenotypic changes between different transformation events and transformed plants are still questionable.

In Malaysia, climate change likes prolonged hot and dry season may induce water deficit. Water deficit gives negative impacts to agricultural activities. In oil palm industry, drought stress severely reduces oil yield and productivity, which can decrease export revenue worth several million Ringgit Malaysia. Oil yield and productivity does not only depend on genetic background of the palm, but it also includes the interaction between palm and the environments (Cha-um et al., 2011). The use of susceptible oil palm seedlings in hot and dry plantation area may also influence the growth and productivity, in which extreme condition may contribute to high injury index and death of the seedlings. Thus, the aims of this study were to observe physiological changes of the oil palm seedlings and to screen and characterize potential SRGs involved in response to drought in oil palm seedlings. The potential SRGs can be used as a molecular marker in plant breeding and genetic engineering to develop abiotic stress tolerant palm. Therefore, the objectives of this study were:

1. To screen potential stress-responsive genes involved in drought stress and determine molecular, biochemical and physiological responses to abiotic stress in oil palm seedlings using *EgDREB1* and other molecular and biochemical markers
2. To isolate and carry out molecular characterization of oil palm *EgDREB1* encoding the complete open reading frame (ORF)
3. To construct a recombinant vector harboring *EgDREB1* and to produce transgenic tomato via *Agrobacterium*-mediated transformation
4. To determine biochemical, physiological and phenotypic changes in response to abiotic stress in non-transgenic and transgenic tomatoes



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