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Research article

Oil palm drought inducible *DREB1* induced expression of DRE/CRT- and non-DRE/CRT-containing genes in lowland transgenic tomato under cold and PEG treatments

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ABSTRACT

Dehydration-responsive element binding (DREB) transcription factor plays an important role in controlling the expression of abiotic stress responsive genes. An intronless oil palm EgDREB1 was isolated and confirmed to be a nuclear localized protein. Electrophoretic mobility shift and yeast one-hybrid assays validated its ability to interact with DRE/CRT motif. Its close evolutionary relation to the dicot NtDREB2 suggests a universal regulatory role. In order to determine its involvement in abiotic stress response, functional characterization was performed in oil palm seedlings subjected to different levels of drought severity and in EgDREB1 transgenic tomato seedlings treated by abiotic stresses. Its expression in roots and leaves was compared with several antioxidant genes using quantitative real-time PCR. Early accumulation of EgDREB1 in oil palm roots under mild drought suggests possible involvement in the initiation of signaling communication from root to shoot. Ectopic expression of EgDREB1 in T1 transgenic tomato seedlings enhanced expression of DRE/CRT and non-DRE/CRT containing genes, including tomato peroxidase (LePOD), ascorbate peroxidase (LeAPX), catalase (LeCAT), superoxide dismutase (LeSOD), glutathione reductase (LeGR), glutathione peroxidase (LeGP), heat shock protein 70 (LeHSP70), late embryogenesis abundant (LeLEA), metallothionine type 2 (LeMET2), delta 1-pyrroline-5- carboxylate synthetase (LePCS), ABA-aldehyde oxidase (LeAAO) and 9-cis- Epoxycarotenoid dioxygenase (LeECD) under PEG treatment and cold stress (4 °C). Altogether, these findings suggest that EgDREB1 is a functional regulator in enhancing tolerance to drought and cold stress.

1. Introduction

Being a sessile organism, plant detects water deficit through its rooting system. The signal perceived by this organ induces communication between the roots and shoots, and the physiological stress alarm is conveyed to the whole plant system (Saradadevi et al., 2014). The primary response involves transmitting signal to the leaves to close their stomata for preventing water loss. The stomatal closure induces reduction in photosynthetic activity which may negatively affect carbohydrate metabolism (Yoon et al., 2014; Shinozaki and Yamaguchi-Shinozaki, 2007). The diminishing carbohydrate metabolism subsequently causes metabolism impairment in plants and results in yield decrement in many food crops (Huber and Huber, 1996; Foyer et al., 1998; Bita and Gerats, 2013). The diminution of photosynthetic activity may also result in an over-reduction of photosynthetic electron chain and excessive production of reactive oxygen species (ROS) such as superoxide anion, hydrogen peroxide hydroxyl radical and singlet oxygen (Praxedes et al., 2006; Rejeb et al., 2014). Overwhelming generation of ROS may hamper normal biological processes and increase internal injury index in plants. However, ROS can also act as an alarm signal to activate plant defense response through specific signal transduction pathways and by having this role, they are no longer

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recognized as toxic byproducts (Reczek and Chandel, 2015).

Expression of a diverse group of genes is induced in plants to adapt and survive under abnormal and extreme conditions. The expressed genes are known as inducible or stress responsive genes (SRGs). The protein products from the expressed SRGs are further classified as functional and regulatory proteins. These include enzymes such as catalase and peroxidase which are involved in metabolic processes. While the regulatory proteins include transcription factors (TFs) such as DREB, bZIP, MYC and MYB as well as protein kinases (e.g. MAP kinase, CDP kinase, receptor protein kinase, ribosomal-protein kinase and transcription-regulation protein kinase) and proteinases (phosphoesterase and phospholipase C). These regulatory proteins are involved in the signal transduction and gene expression regulations (Yamaguchi-Shinozaki and Shinozaki, 2005; Agarwal et al., 2006; Zhou et al., 2010). The TFs perceive stress signal from the environment and further bind to *cis*-acting element located in the SRGs' promoter sequence. This activity enables the SRGs to be expressed resulting in production of stress-related proteins and metabolites. These compounds are further utilized by plants for survival and adaptation under extreme conditions as well as protection of the cells from the stresses (Xiong et al., 2002; Shinozaki et al., 2003; Mahajan and Tuteja, 2005; Kosova et al., 2011).

Dehydration responsive element binding (DREB) protein belongs to an important group of plant-specific transcription factors. It contains a highly conserved AP2/ERF domain with approximately 58–70 amino acids (Gutha and Reddy, 2008; Zhao et al., 2010; Zhou et al., 2010). The domain consists of three-stranded β-sheets and one α-helix running almost parallel to the β-sheet. It interacts with DNA via Arg and Trp residues located in the β-sheet (Lata and Prasad, 2011). DREB family is well known to be interacting with the DRE located in the promoter sequence of the SRGs. The DRE/CRT motif has a 6 bp conserved core sequence (5′-ACCGAC-3′). DRE/CRT was first identified in the promoter of the drought-responsive gene *rd29A*. The *rd29A* gene expression was reported to be up-regulated during drought, high salinity and low temperature, but not by the abscisic acid (ABA). The up-regulation is controlled by the interaction of DREB with the DRE element in the *rd29A* promoter (Lata and Prasad, 2011).

In *Arabidopsis*, DREBs are further classified into six subgroups (A-1 until A-6) based on structural characteristics. The different subgroups of DREBs are believed to play a different role in plants (Sakuma et al., 2002). The DREB1 proteins belong to the A-1 subgroup, are involved in regulating cold-responsive gene expression. It has a NLS consensus PKRPAGRTKFRETRHP that differentiates these proteins from other AP2/ERF proteins. Moreover, the DSAW motif at the end of the AP2/ERF domain and LWSY motif at the end of the C-terminal are conserved in most DREB1-type proteins (Agarwal et al., 2006; Yang et al., 2009; Li et al., 2014). Most reports revealed that the DREB1 is involved in a low temperature transduction pathway, while, DREB2 subfamily are more accountable in the dehydration transduction pathway. Both pathways rely on ABA-independent signaling system. Also, DREB2 proteins which belong to the A-2 subgroup have been shown to be involved in situations of dehydration and high salinity (Zhao et al., 2012). In a recent paper, Dossa et al. (2016) reported on the role of DREB1 genes in response to drought in sesame.

Xin et al. (2011) reported that among the DREB TFs, DREB1 was the most studied in the last 20 years. The various studies indicated that DREB1 TFs are promising candidates for the development of stress-tolerant plants by genetic manipulation at the transcriptional level. Sequence analysis of *DREB1* genes showed that the genes are intronless, and their duplication may produce a small multigene family during species evolution (Gutha and Reddy, 2008). The *DREB1* genes have been proven to regulate downstream genes in response to low and high temperatures through ABA dependent and independent pathways. However, most of the DREBs are classified as ABA-independent proteins in which their activity is not subjected to accumulation of ABA.

Oil palm is a major oil crop which is severely affected by drought stress due to abnormal weather condition in recent years. The involvement of DREB1 in cold stress tolerance is well documented, however information about its role in drought stress response is lacking. Understanding participation of *DREB1* in stress signaling pathway may provide useful information for designing strategies to overcome abiotic stresses in oil palm. Functional characterization was performed on monocotyledonous oil palm *DREB1* gene, designated as *EgDREB1* in response to drought and cold stress. We profiled the expression of *EgDREB1* in vegetative tissues of oil palm seedlings exposed to different severity of drought. Functional analysis was also carried out in the lowland transgenic tomato over-expressing *EgDREB1* under PEG and cold treatments.

2. Materials and methods

2.1. Plant materials

Elaeis guineensis Jacq. var. *tenera* spear leaves obtained from University Agriculture Park, Universiti Putra Malaysia (UPM) were cleaned, cut and weighed into two gram portions then frozen in liquid nitrogen and stored at −80 °C. The genomic DNA from this tissue was used for isolation of *DREB1*. Three-month-old Golden Hope 500 series (GH500) of oil palm seedlings were bought from the Sime Darby R & D Centre, Banting, Selangor and left for 2 months to acclimatize in the glasshouse at UPM before subjecting them to drought treatments. They were kept at ambient temperature, watered daily and given fertilizer fortnightly. The seedlings were produced from seed germination process. The GH500 is a cross-breed product of Elite Dura and second generation of Pisifera, BM119. Genotype of these oil palm seedlings have not been reported to be tolerant against drought stress. But, they have been reported as elite oil palm which potentially produce fresh fruit bunch (FFB) exceeding 40 metric ton/hectare and oil extraction rate exceeding 31% (Golden Hope, 2005).

Tomato (*Lycopersicon esculentum* Mill cv. MT1) seeds purchased from the Unit of Planting Materials, Seed and Livestock Breed, Malaysian Agricultural Research and Development Institute (MARDI), Serdang, Selangor. This cultivar has not been reported to be tolerant against abiotic stresses. The seeds were surface sterilized with 95% ethanol for 30 s. Then, the seeds were soaked twice in 5% sodium hypochlorite containing two drops of Tween-20 for 10 min and finally washed with sterile distilled water four to five times until the foam was completely removed. The sterile seeds were put on the sterile petri dish under layered with sterile filter paper. The seeds were germinated on Murashige and Skoog (1962) medium supplemented with B5 vitamins (MSB5) (Gamborg et al., 1968), 30 g sucrose, 2.75 g gelrite and pH 5.8. All cultures were inoculated under 16/8 h (day/night) photoperiod with a photon flux density of 150 μmol/m^2/s at 24 ± 2 °C.

2.2. Drought treatment of the oil palm seedlings and physiological analysis

For drought treatment, 5-month-old acclimatized oil palm seedlings were used. The water was withheld from the seedlings for 7, 14, 21, 28 and 35 days, except the control seedlings. The control seedlings were watered every day. The experiment was carried out using completely randomized design (CRD). Each treatment comprised 5 replicates with 5 plants per replicate.

CO_2 assimilation (A), leaf transpiration (E) and stomatal