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Overexpression of oil palm *EgDREB1* in tomato decreased fruit size and produced parthenocarpic fruits

A.M. AZZEME[1], S.N.A. ABDULLAH[2,3]*, M.A. AZIZ[3], and P.E. MEGAT WAHAB[4]

Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, University Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia[1]
Laboratory of Plantation Science and Technology, Institute of Plantation Studies, University Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia[2]
Department of Agriculture Technology, Faculty of Agriculture, University Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia[3]
Department of Crop Sciences, Faculty of Agriculture, University Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia[4]

Abstract

Drought-responsive element binding (DREB) is involved in the regulation of stress-responsive gene expressions in plants through abscisic acid (ABA)-independent pathway. In this study, constitutive expression of oil palm (*Elaeis guineensis*) *EgDREB1* driven by double strength cauliflower mosaic virus 35S promoter in tomato (*Solanum lycopersicum*) reduced seed number, produced parthenocarpic fruits, changed morphology of leaves, and increased root biomass of transgenic plants. Early flowering and fruiting of the transgenic lines were observed in the culture vessels. *EgDREB1* was specifically expressed in the fruits and its expression was not detected in vegetative tissues (leaves and roots). Altered expression of several endogenous tomato genes involved in the biosynthesis of phytohormones including jasmonic acid, ethylene, auxin, cytokinin, gibberellin (GA) and ABA were observed compared to wild type plants. The expression of *AP2-like-ethylene transcription factor* (*LeAP2*), *allene oxide synthase* (*LeAOS*), *allene oxide cyclase* (*LeAOC*), *aminocyclopropane-1-carboxylic acid synthase* (*LeACS*), *1-aminocyclopropane-1-carboxylate oxidase 1* (*LeACO*), *auxin responsive factor 8* (*LeARF8*), *auxin/indole-3-acetic acid* (*LeAux/IAA*), *cytokinin oxidase/dehydrogenase-like* (*LeSlCKX1*), *adenylate isopentenyltransferase* (*LeSlIPT1*), *gibberellin 2-oxidase 2* (*LeGa2ox2*), *gibberellin 20-oxidase 4* (*LeGa20ox4*) and *ABA-aldehyde oxidase* (*LeAAO*) were different in fruits with reduced seed number compared to parthenocarphic fruits. These results suggest that their expression has significant effects on fruit development in transgenic tomato. *EgDREB1* may mediate the expression of some of these genes as dehydration-responsive element binding (DRE) motif were found in their promoter sequences. These data indicate that the EgDREB1 controls fruit development in trsngenic plants by regulating the expression of hormone-associated genes.

Additional key words: abscisic acid, auxin, cytokinin, ethylen, gibberelin, *Elaeis guineensis, Solanum lycopersicum*, transgenic plants.

Introduction

Parthenocarpic fruit develops without undergoing normal fertilization or through embryo abortion. Natural occurrence of parthenocarpy is associated with elevated content of several phytohormones in the ovary. These include auxins, cytokinins, and gibberellins (GAs) which are involved in early seed and fruit development. Other phytohormones such as ABA and ethylene are vital for seed maturation and fruit ripening (Fos *et al*. 2000, Martinelli *et al.* 2009, Bonghi *et al.* 2011, Li *et al.* 2011). In agriculture, this trait is usually observed when the plants

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Abbreviations: ABA - abscisic acid, CaMV - cauliflower mosaic virus; DREB - drought-responsive element binding; EgDREB1 - dehydration-responsive element-binding; GA - gibberellin; LeAAO - ABA-aldehyde oxidase; LeACO1 - 1-aminocyclopropane-1-carboxylate oxidase 1; LeAOC - allene oxide cyclase; LeACS - aminocyclopropane-1-carboxylic acid synthase; LeAOS - allene oxide synthase; LeAP2 - AP2-like-ethylene transcription factor; LeARF8 - auxin response factor 8; LeAUX/IAA - auxin/indole-3-acetic acid; LeCYC - cyclophilin; LeGA20ox4 - gibberellin 20-oxidase 4; LeGAPDH - glyceraldehyde-3-phospahte dehydrogenase; LeGA2ox2 - gibberellin 2-oxidase 2; LeSlCKX1 - cytokinin oxidase/dehydrogenase-like; LeSlIPT1 - adenylate isopentenyltransferase; LeTUB - tubulin; SRG - stress-responsive gene; WT - wild type.

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* Corresponding author; fax: (+60) 389381612, e-mail: snaa@upm.edu.my

are grown in unfavourable conditions such as low or high temperatures, low or high humidity, and low irradiance that may interfere with pollen production, anther dehiscence or pollination. The absence of seeds in fruits can increase fruit quality and fruit shelf-life which enhance consumer preference (Rojas-Gracia, *et al.* 2017, Ueta *et al.* 2017).

Parthenocarpy can also be induced artificially through the application of natural and synthetic phytohormones like GAs, cytokinins, and auxins during ovary development (Osborne and Went 1953, Ficcadenti *et al.* 1999, Dhatt and Kaur 2016). Liu *et al.* (2018) showed that application of GA_4 or GA_7 can induce parthenocarpy in pear, but the use of GA_3 did not produce parthenocarpic trait in pear fruit. However, application of GA_3 produced parthenocarpic grape, tomato, custard apple (Dos Santos 2016), and *Siraitia grosvenorii* (Tu *et al.* 2017). The application of cytokinin like compound (N_1-(2-chloro-4-pyridyl)-N_3-phenylurea; CPPU) induced parthencarpic teasle gourd (Rasul *et al*. 2008), cucumber (Qian *et al.* 2018) and tomato (Ding *et al.* 2013) while treatment with auxin induced parthenocarpic tomato (Koshioka *et al.* 1994, Alabadi *et al.* 1996, Ramin, 2003, Serrani *et al.* 2007).

The involvement of a number of genes in inducing parthenocarpic fruit has been reported in tomatoes. The *parthenocarpic fruit* (*pat*) genes are involved in the formation of stable parthenocarpic tomato. There are eight *pat* genes characterized from five types of tomato mutants. The parthenocarpic genotypes Soressi and Montfavet191 are controlled by a single recessive *pat*, while Severianin is controlled by a single recessive *pat-2* and MPK-1 is controlled by a single recessive *Pat-k*. The RP75/59, IL5-1, and IVT-line1 tomatoes are controlled by the recessive genes *pat3*/*pat4*, p*at4.1*/*pat5.1*, and *pat4.2*/*pat9.1*, respectively (Takisawa *et al.* 2018).

Transcriptional regulatory proteins have been implicated in parthenocarpy formation. The *AUXIN RESPONSE FACTOR8 (ARF8)* is involved in inhibiting carpel development in the absence of fertilization and the generation of signals required to initiate fruit and seed development (Goetz *et al.* 2006). In natural parthenocarpic mutant of eggplant, *SmARF8* was down-regulated in the buds. Transgenic RNAi involving this gene in eggplant exhibited parthenocarpy in unfertilized flowers. Whereas, overexpression of *SmARF8* in *Arabidopsis* also induced parthenocarpy (Du *et al.* 2016). Downregulation of a gene encoding INDOLE-3-ACETIC ACID INDUCIBLE 9 (IAA9) transcription factor also participated in the development of parthenocarpic tomato. The IAA9 was also reported to be involved in controlling leaf formation of parthenocarpic plant (Wang *et al.* 2005). Hence, the involvement of auxin signaling pathway genes is important in regulating parthenocarpic fruit development.

Drought-responsive element binding 1 (DREB1) belongs to AP2/ERF superfamily of transcription factors and it has been reported to be involved in regulating stress responsive genes (*SRGs*) expression through ABA-independent pathway. The protein binds to dehydration-responsive element/C-repeat (DRE/CRT) motif located in *SRGs* promoters and activates *SRGs* expression (Zhang *et al.* 2014). Involvement of *DREB1* gene in controlling *SRG* expression has been widely reported. The gene has the ability to enhance stress tolerance to drought, freezing and salinity based on the reports from various plants including tomato (Azzeme *et al.* 2017), wheat (Wang *et al.* 2006), rice (Ito *et al.* 2006), potato (Bouaziz *et al.* 2012), and barley (Soltesz *et al.* 2013).

Our previous study revealed that the overexpression of the oil palm (*Elaeis guineensis* Jacq.) *EgDREB1* in PEG- and cold-treated lowland tomato seedlings upregulated the expression of tomato genes like *peroxidase*, *ascorbate peroxidase*, *glutathione peroxidase*, *catalase*, *heat shock protein 70*, *late embryogenesis abundant*, *metallothionein type 2*, *delta 1-pyrroline-5-carboxylate synthetase* and *adenylate isopentenyltransferase*. These genes contain DRE/CRT motif in their promoter sequence (Azzeme *et al.* 2017). Overexpression of citrus *DREB* in tomato, however, produced smaller fruit size and induced accumulation of primary metabolites (Nishawy *et al.* 2015). In this study, overexpression of *EgDREB1* also led to the production of smaller fruit size, but in addition, reduction in seed number and production of parthenocarpic fruit were observed. From these findings we further profiled the expression of *EgDREB1* and hormone-associated genes in transgenic fruits with reduced seed number and parthenocarpic characteristics to further understand the involvement of these genes in fruit development.

Materials and methods

Plants and *Agrobacterium*-mediated transformation: Tomato (*Solanum lycopersicum* Mill cv. MT1) seeds were purchased from the Malaysian Agricultural Research and Development Institute (MARDI), Serdang and kept at 4 °C. The seeds were further germinated on Murashigae and Skoog (MS) media as described by Azzeme *et al.* (2017).

Two hundred of 7- to 10-d-old cotyledons were cut and co-cultivated with *Agrobacterium tumefaciens* strain LBA4404 harboring pMDC-EgDREB1 and pMDC(-32) (empty vector) as described by Azzeme *et al.* (2017). The vector map of pMDC-EgDREB1 and pMDC(-32) is described in Fig. 1 Suppl.

Verification and regeneration of transgenic plants: Total genomic DNA was isolated from putative transgenic leaves using *DNeasy* kit (*Qiagen*, Hilden, Germany). The putative transgenic plants with pMDC(-32) were verified by PCR with CaMV 35S promoter primers (forward primer: 5'-GCTCCTACAAATGCCATCA-3'; reverse primer: 5'-GATAGTGGGATTGTGCGTCA-3'), while transgenic lines with EgDREB1 were verified by *hygromycin resistance gene* (*hptII*) primers (forward primer: 5'-ATTTCTTTGCCCTCGGACGAGTGCT-3'; reverse primer: 5'-AAAGCCTGAACTCACCGCGACGTCT-3').

Six independent transgenic lines harboring *Elaeis guineensis DREB1* (*EgDREB1*) (named as EgDREB1-L5, EgDREB1-L9, EgDREB1-L13, EgDREB1-L1, EgDREB1-L3, and EgDREB1-L10), transgenic plant harboring empty vector [named as pMDC(-32)] and WT plant were grown in the greenhouse, Universiti Putra Malaysia, at natural conditions (a temperature 28 - 30 °C) (Azzeme et al. 2017). The empty vector plant harbouring only a pMDC-32 vector and not the *ccd*B and *EgDREB1* genes. The six independent EgDREB1 transgenic lines, pMDC(-32) plant, and WT plant were washed carefully with sterile distilled water to remove excess *Gelrite* and subsequently transferred to the pots filled with superfine peat soil (*Holland*, *AG Substrate*, Melaka, Malaysia). We studied T_0 plants because the parthenocarpic fruits did not have seeds to grow for the next generation. The plantlets were covered with transparent plastic to maintain high air humidity. The plantlets were grown in a controlled growth chamber at a temperature of 24 °C, a 16-h photoperiod, and an irradiance of 150 μmol m^{-2} s^{-1} until they were able to survive cultivation in the above mentioned greenhouse. The plants were fertilized every two weeks with N + P + K (15:30:15). The leaf morphology, fruit number, and seed number of WT, pMDC(-32), and EgDREB1 transgenic lines were recorded and ripe fruits (40 d after anthesis) were collected for analyses.

Expression profile of genes potentially involved in the development of parthenocarpic fruits: The fruits, leaves, and roots (500 mg) from WT and transgenic plants pMDC(-32) and EgDREB1 were ground into fine powder in liquid nitrogen with mortar and pestle. The total RNA was isolated using *Sepasol RNA 1 Super G* (*Nacalai Teque*, Kyoto, Japan). The DNAse I treatment was carried out as described by Azzeme *et al*. (2016). The first-strand cDNA was synthesized from total RNA using *SuperScript*™ *III* first-strand synthesis system for reverse-transcription PCR (*Invitrogen*, Carlsbad, USA) (Azzeme *et al.* 2017). All primers (Table 1 Suppl.) for qPCR were designed by using *Primer3* (*v.0.4.0*) (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). In this study, three endogenous controls (*GAPDH*, *cyclophilin*, and *β-tubulin*) from tomato were tested across the samples. The qPCR was performed using *Power SYBR® Green PCR Master Mix* (*Applied Biosystem*, Foster City, USA) with three technical replicates per sample. All tomato genes [*allene oxide synthase* (*LeAOS*), *allene oxide cyclase* (*LeAOC*), *AP2-like-ethylene transcription factor* (*LeAP2*), *aminocyclopropane-1-carboxylic acid synthase* (*LeACS*), *1-aminocyclopropane-1-carboxylate oxidase 1* (*LeACO*), *auxin responsive factor 8* (*LeARF8*), *auxin/indole-3-acetic acid* (*LeAux/IAA*), *cytokinin oxidase/dehydrogenase-like* (*LeSlCKX1*), *adenylate isopentenyltransferase* (*LeSlIPT1*), *gibberellin 2-oxidase 2* (*LeGA2ox2*), *gibberellin 20-oxidase 4* (*LeGA20ox4*) and *ABA-aldehyde oxidase* (*LeAAO*)] were identified from the National Centre for Biotechnology Information (*NCBI*) (http://www.ncbi.nlm.nih.gov/). The calibrator used to quantify *EgDREB1* expression in fruit was fruit from transgenic line *EgDREB1-L3* because it had the highest Ct value. Meanwhile, the genes of wild type tomato fruit were used as the calibrators to quantify the tomato genes in transgenic fruits.

The promoter sequences of hormone-associated genes were downloaded from the tomato genome database *SOL Genomics Network* (http://solgenomics.net/) (Bombarely *et al.* 2011). The promoter sequences were further analyzed using *PLACE* (http://www.dna.affrc.go.jp/PLACE/).

Results

In our previous study (Azzeme *et al.* 2017), we successfully isolated and characterized EgDREB1 as a plant transcription factor. In this study, six independent transgenic lines harboring *EgDREB1* were successfully produced. They showed different growth characteristic compared to the controls [WT and empty vector, pMDC (-32) plants]. Therefore, phenotypic analysis was carried out.

As shown in Fig. 2 Suppl, transgenic lines produced *in vitro* flowers and fruits after transferring them to rooting medium (full strength MS basal salts containing 1 mg dm^{-3} IAA, 6 mg dm^{-3} hygromycin and 150 mg dm^{-3} timentin). They showed slower growth rate than that of WT and pMDC(-32) plants. A similar observation was observed when they were transferred to the soil and grown in a growth chamber at 24 ºC ± 2 (Fig. 3 Suppl.). After 30 d, the transgenic and control plants were transferred to the greenhouse and further grown at ambient temperature of 28 - 30 ºC. Interestingly, the transgenic lines showed faster growth rate than that of control plants, but slower production of flowers and fruits. The leaves of pMDC (-32) plants and WT plants did not exhibit any differences from each other (Fig. 4 Suppl.). However, the leaves of EgDREB1 plants exhibited rolling and curling inwards (Fig. 4 Suppl.). Root mass of transgenic lines also increased compared to that of WT and pMDC(-32) plants (Fig. 5 Suppl.).

Moreover, the transgenic lines also produced parthenocarpic fruits (EgDREB1-L5 and EgDREB1-L9) and fruits with reduced seed numbers (EgDREB1-L1, EgDREB1-L3, EgDREB1-L10, and EgDREB1-L13) (Fig. 1, Table 1). The transgenic lines EgDREB1-L5 and EgDREB1-L9 did not produce seeds, while EgDREB1-L1, EgDREB1-L3, EgDREB1-L10, and EgDREB1-L13 produced 33, 44, 39, and 30 seeds, respectively, which is lower than wild type and pMDC(-32) plants.

Since the transgenic fruits showed decreased seed number when compared to WT and pMDC(-32) plants,

Table 1. The number of seeds from wild type, pMDC(-32), and EgDREB1 plants. Means of 10 fruits.

Plant type	Number of seeds
Wild type	720
MDC(-32)	700
EgDREB1-L5	0
EgDREB1-L9	0
EgDREB1-L1	33
EgDREB1-L3	44
pMDC-EgDREB1-L10	39
pMDC-EgDREB1-L13	30

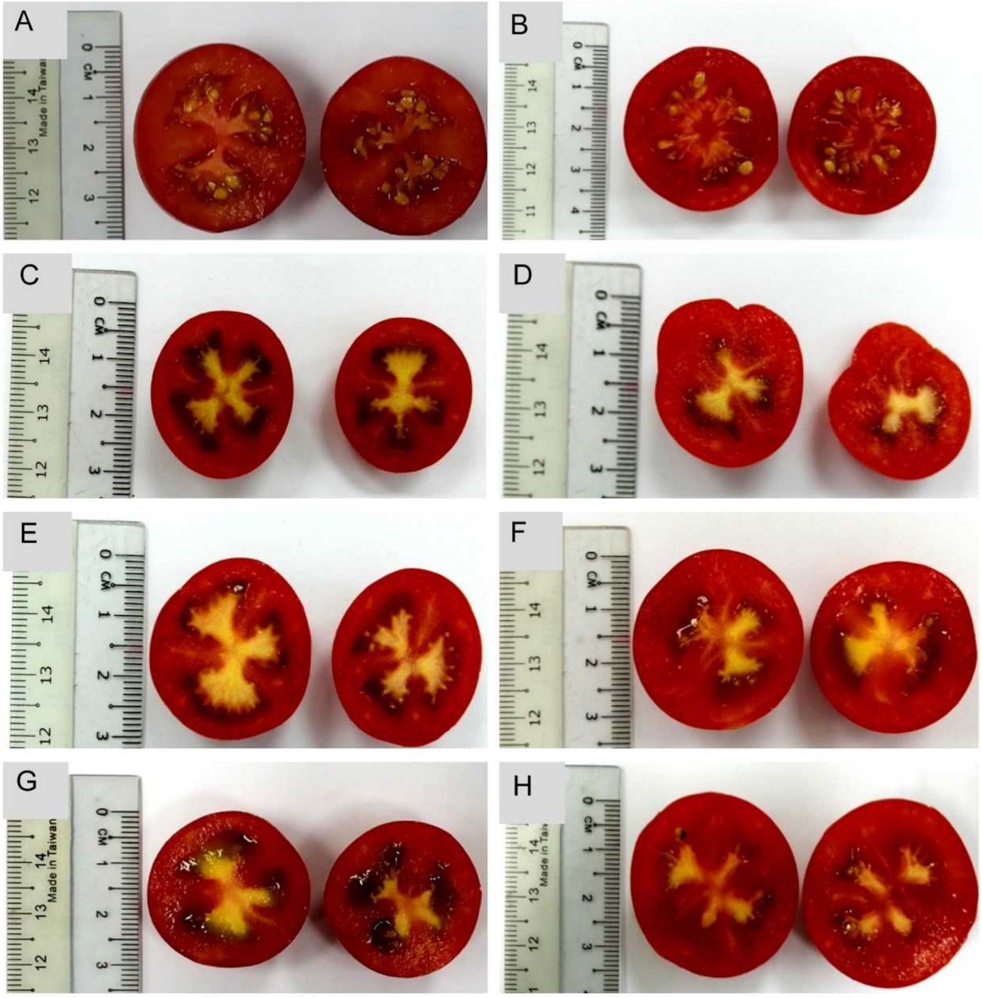

Fig. 1. Phenotypes of tomato fruits: *A* - wild type, *B* - pMDC(-32), *C* to *H* - transgenic lines EgDREB1-L5, EgDREB1-L9, EgDREB1-L13, EgDREB1-L1, EgDREB1-L3, and EgDREB1-L10, respectively.

gene expression analysis was carried out in fruits. The calibrator used to analyze the expression *EgDREB1* was the EgDREB1-L3 as it has the highest C_T value (Fig. 2). The expression of *EgDREB1* in EgDREB1-L5, EgDREB1-L9, EgDREB1-L1, EgDREB1-L10, and EgDREB1-L13 was 4.76-fold, 9.55-fold, 4.35-fold, 3.15-fold and 2.39-fold, respectively relative to the expression of EgDREB-L3.

Furthermore, because EgDREB1 is a transcription factor which can be involved in mediating the expression of phytohormone-associated genes, here we further profiled the expression of jasmonic acid- (*LeAOS* and *LeAOC*) ethylene- (*LeAP2*, *LeACS* and *LeACO*), auxin- (*LeARF8*, *LeAux/IAA*), cytokinin- (*LeSlCKX1* and *LeSlIPT1*), GA- (*LeGA2ox2*, *LeGA20ox4*), and ABA- (*LeAAO*) associated genes in transgenic fruits (Fig. 3).

The expression of *LeAP2* was expressed in a distinctive manner in the fruits of different transgenic lines (Fig. 3*A*). The *LeAP2* was up-regulated in fruits of both parthenocarpic lines. The fold increase compared to wild type was 4.16 in EgDREB1-L5 and 4.06 in EgDREB1-L9. However, the expression of *LeAP2* was down-regulated in EgDREB1-L1 (0.95-fold), EgDREB1-L3 (0.70-fold), EgDREB1-L10 (0.86-fold), and EgDREB1-L13 (0.99-fold). The expression of key genes involved in ethylene biosynthetic pathway, *LeACS* (Fig. 3*B*) and *LeACO* (Fig. 3*C*) was regulated in a different manner between parthenocarpic transgenic fruits and those with reduced seed number. The expression of *LeACS* was up-regulated in EgDREB1-L5 and EgDREB1-L9 fruits with the fold change of 2.94 and 9.55, respectively, compared to WT. But, in the EgDREB1-L1, EgDREB1-L3, EgDREB1-L10, and EgDREB1-L13 the *LeACS* expression was down-regulated to 0.64-fold, 0.78-fold, 0.97-fold, and 0.90-fold, respectively. The expression of *LeACO* was up-regulated in EgDREB1-L5 (1.40-fold), EgDREB1-L9 (3.37-fold), EgDREB1-L10 (1.69-fold) and EgDREB1-L13 (1.50-fold) while the gene was down-regulated in EgDREB1-L1 (0.35-fold) and EgDREB1-L3 (0.97-fold).

The participation of jasmonic acid in the development of fruit set was indicated by the expression of the *LeAOS* and *LeAOC* (Fig. 3*D* and Fig. 3*E*, respectively). The expression of *LeAOS* was up-regulated in both parthenocarpic lines, EgDREB1-L5 (8.31-fold) and EgDREB1-L9 (4.46-fold) compared to WT. The expression of *LeAOS* in

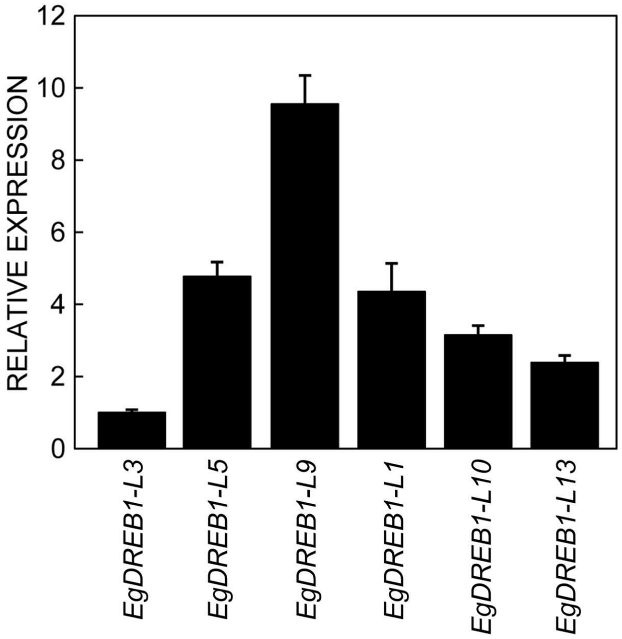

Fig. 2. Relative expressions of *Elaeis guineensis DREB1* (*EgDREB1*) in transgenic tomato fruits. Means ± SEs, $n = 3$. Fold changes in expressions in comparison with the expression of line *EgDREB1-L3* (having the highest Ct value), which was set to 1.

Table 2. The copy number of a dehydration-responsive element (DRE) motif and its location in the tomato genes used in expression analysis. *LeARF8 - auxin responsive factor 8*; *LeAP2 - AP2-like-ethylene transcription factor*; *LeACS - aminocyclopropane-1-carboxylic acid synthase*; *LeGA2ox2 - gibberellin 2-oxidase 2*; *LeSlIPT1 - adenylate isopentenyltransferase*; (+) - sense strand; (-) - antisense strand.

Name of gene	Copy number	Location
LeARF8	2	4603 (+), 4603 (-)
LeAP2	2	800 (+), 1602 (-)
LeACS	2	4946 (+), 4946 (-)
LeGA2ox2	2	3 (+), 4580 (+)
LeSlIPT1	1	2080 (-)

transgenic lines with reduced seed number was down-regulated by about 0.11-fold in EgDREB1-L1, 0.30-fold in EgDREB1-L3, 0.90-fold in EgDREB1-L10, and 0.93-fold in EgDREB1-L13. The expression of *LeAOC* was up-regulated in the transgenic lines of parthenocarpic fruits by 1.19-fold in EgDREB1-L5 and 1.81-fold in EgDREB1-L9. However, the expression of *LeAOC* was down-regulated about 0.41-fold in the fruit of EgDREB1-L1, 0.84-fold in EgDREB1-L3, 0.41-fold in EgDREB1-L10, and 0.39-fold in EgDREB1-L13.

The expression of *LeARF8* (Fig. 3*F*) was up-regulated in all transgenic fruits compared to that of WT (3.97-fold in EgDREB1-L5, 4.16-fold in EgDREB1-L9, 2.13-fold in EgDREB1-L1, 2.56-fold in EgDREB1-L3, 2.94-fold in EgDREB1-L10, and 3.62-fold in EgDREB1-L13). The expression of *LeAUX/IAA* was also up-regulated in EgDREB1-L5 (6.30-fold), EgDREB1-L9 (7.41-fold), EgDREB1-L1 (1.81-fold), EgDREB1-L10 (1.37-fold) and EgDREB1-L3 (1.50-fold) fruits except for EgDREB1-L3 (0.64-fold) whose expression was down-regulated (Fig. 3*G*).

The expression of *LeSlCKX1* was up-regulated by about 4.46-fold in the fruit of EgDREB1-L5, 7.24-fold in EgDREB1-L9, 1.69-fold in EgDREB1-L1, 41.90-fold in EgDREB1-L3, 49.26-fold in EgDREB1-L10 and 7.41-fold in EgDREB1-L13 (Fig. 3H). The fold change in expression was also investigated for *LeSlIPT1* (Fig. 3*I*). The mRNA of *LeSlIPT1* accumulated about 10-fold more in both parthenocarpic transgenic lines (EgDREB1-L5 and EgDREB1-L9) than in WT fruits. In EgDREB1-L1, EgDREB1-L3, EgDREB1-L10, and EgDREB1-L13, the expression was up-regulated by about 2.39-fold, 2.08-fold, 1.14-fold, and 6.30-fold, respectively.

The change in the expression of GA biosynthesis genes, *LeGA2ox2* (Fig. 3J) and *LeGA20ox4* (Fig. 3K) was also investigated. Expression of *LeGA20ox2* was slightly down-regulated in the fruit of EgDREB1-L5 (0.93-fold), EgDREB1-L9 (0.84-fold), EgDREB1-L1 (0.05-fold), EgDREB1-L3 (0.03-fold), EgDREB1-L10 (0.12-fold), and EgDREB1-L13 (0.46-fold) compared to the WT. Whereas, the expression of *LeGA20ox4* in the transgenic fruit was up-regulated by 7.76-fold (EgDREB1-L5), 11.76-fold (EgDREB1-L9), 6.02-fold (EgDREB1-L1), 20-fold (EgDREB1-L3), 11.23-fold (EgDREB1-L10), and 7.24-fold (EgDREB1-L3).

The involvement of ABA was observed by determining the expression profile of *LeAAO* (Fig. 3*L*). The expression of *LeAAO* was up-regulated about 3.62-fold, 2.08-fold, 2.33-fold, 5.49-fold, 2.44-fold, and 3.88-fold in fruits of EgDREB1-L5, EgDREB1-L9, EgDREB1-L1, EgDREB1-L3, EgDREB1-L10, and EgDREB1-L13 transgenic lines, respectively.

To further confirm the interaction of EgDREB1 with DRE/CRT motif located in the promoter region of each hormone associated genes, the promoter sequences were downloaded and analyzed. Among the genes, *LeARF8, LeAP2, LeACS, LeGA2ox2*, and *LeSIIPT1* contained DRE/CRT motif in their promoter (Table 2). It is suggested that the *EgDREB1* is involved in regulation of expression of these genes during fruit formation by direct binding with DRE/CRT motif located in their promoter.

Discussion

DREB transcription factors are well known to be involved in the regulation of abiotic stress responses and plant development (Rejeb *et al.* 2014) but the downstream genes regulated by DREB in ripening fruits are not well understood. In the present study, two independent transgenic lines with parthenocarpic fruits and four independent transgenic lines with reduction in seed number produced by ectopic expression of oil palm *EgDREB1* were analyzed. The high expression of *EgDREB1* in the

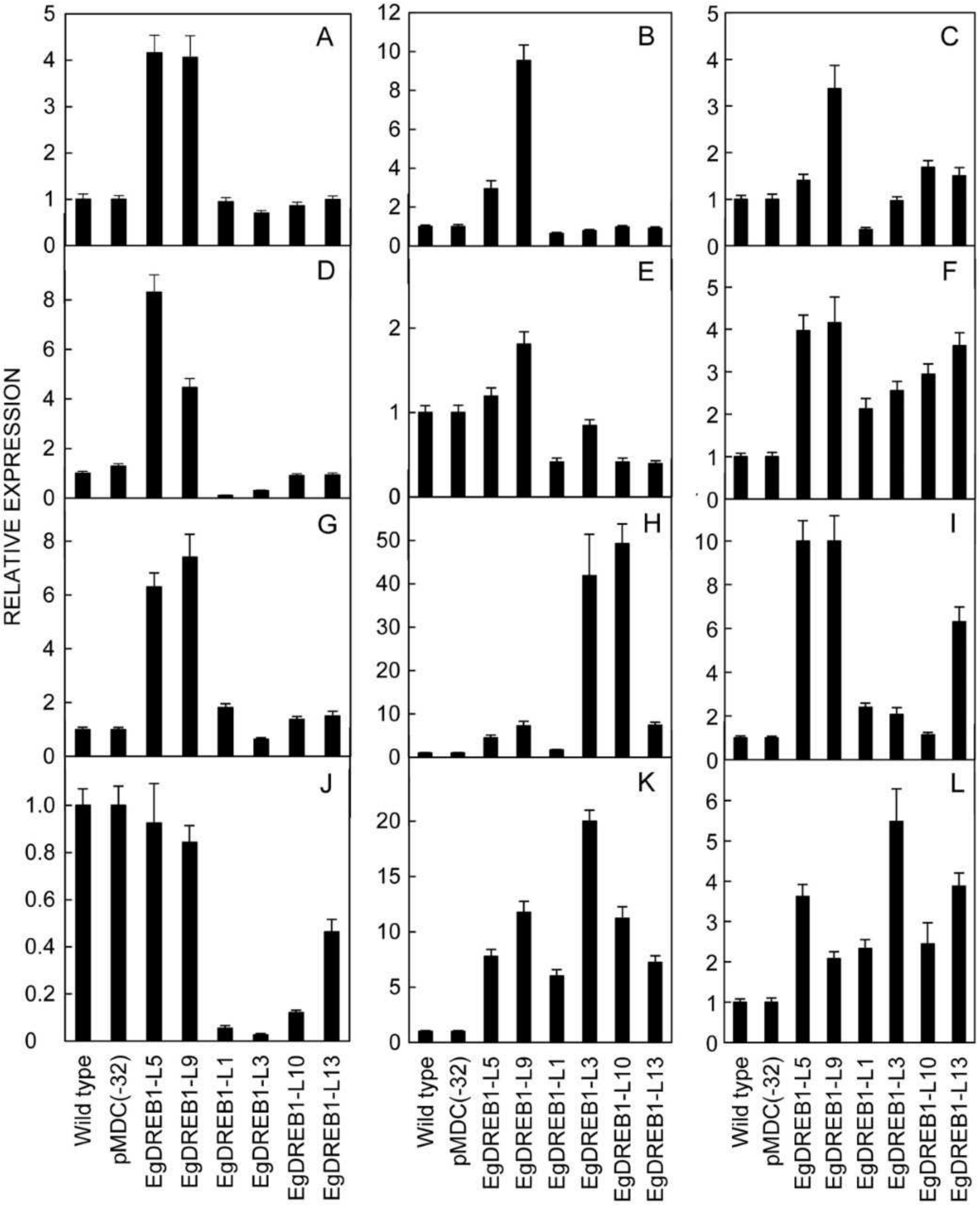

Fig. 3. Expression patterns of genes potentially involved in the development of parthenocarpic tomato fruits: *A* - *AP2-like-ethylene transcription factor*, *B* - *aminocyclopropane-1-carboxylic acid synthase, C* - *1-aminocyclopropane-1-carboxylate oxidase 1*, *D* - *allene oxide synthase*, *E* - *allene oxide cyclase*, *F* - *auxin responsive factor 8*, *G* - *auxin/indole-3-acetic acid*, *H* - *cytokinin oxidase/dehydrogenase-like*, *I* - *adenylate isopentenyltransferase*, *J* - *gibberellin 2-oxidase 2*, K - *gibberellin 20-oxidase 4*, and *L* - *ABA-aldehyde oxidase*. Means ± SEs, *n* = 3. Fold changes in expressions in comparison with the expression of the wild type, which was set to 1.

fruits of transgenic tomato suggests the involvement of *EgDREB1* in controlling fruit development which determines fruit size and seed numbers. The seed number formation influences the final size and mass of the fruit (Martinelli *et al.* 2009). Reduction of fruit size was also observed when *Citrus grandis DREB* was overexpressed in tomato (Nishawy *et al.* 2015).

The use of CaMV35 promoter has been reported to increase the chances of transcriptional inactivation in certain tissues (Stam *et al.* 1997) which could explain the non-detectable expression of *EgDREB1* in roots and leaves. Nevertheless, the increase in root biomass and repression of extension of the leaves is similar to the observation by Li *et al.* (2018), when *ZmDREB4.1* was constitutively expressed in tobacco. This suggests that the vegetative tissues of the transgenic tomatoes were experiencing the effects of *EgDREB1* expression even though the expression was not detected in this study, possibly due to the very low abundance or rapid degradation of the transcripts.

The growth retardation of transgenic plants was observed when they were acclimatized in the growth room suggesting that the ectopic expression of *EgDREB1* has interfered with the GA pathway in the transgenic tomatoes. However, the semi-dwarfism effect was recovered by transferring the transgenic plants to glasshouse with direct sunlight indicating that expression of some of the GA biosynthetic genes were modified in the *EgDREB1* transgenic lines with the change in irradiance. The transgenic plants could be experiencing a change in GA metabolism during the transition of the plants from the growth room with irradiance of 150 µmol m^{-2} s^{-1} to direct full sun together with rise temperature from about 24 ºC and 28 - 30 ºC, respectively. The dwarfism phenotype in *AtDREB1B* tomatoes was successfully prevented by exogenous application of GA (Hsieh *et al.* 2002a,b). The dwarf phenotype was also discovered in *Arabidopsis* over-expressing *DREB1A* (Kasuga *et al.* 1999). Constitutive expression of *TaDREB2* and *TaDREB3* produced transgenic barley with slower growth, less tillers, and delayed flowering under well-watered condition (Morran *et al.* 2011).

The upregulation of *EgDREB1* in transgenic fruits appeared to hamper seed production. Therefore, quantification of the expression of hormone-associated genes involved in fruit development was carried out. Among the ethylene-associated genes with altered expression, only *LeAP2* contains DRE/CRT element in its promoter. Hence, the dimerization of EgDREB1 with other proteins might have occurred in regulating the expression of the other genes as reported by Azzeme *et al*. (2017). LeAP2 may also be involved in fruit ripening and the regulation of auxin signaling pathway. Up-regulation of *AP2* gene to control expression of ethylene- and auxin- associated genes in tomato was also reported by Pasaresi *et al*. (2014). Martinez *et al*. (2013) reported that ethylene-associated genes were highly expressed in the ovary of tomato flowers at anthesis in parthenocarpic genotypes, but they were down-regulated after fruit set. In the present study, down-regulation of *LeACS* was observed in all fruits with reduced seed numbers, while expression of *LeACO* was down-regulated in two lines of transgenic fruit (EgDREB1-L1 and EgDREB1-L3). The downregulation and up-regulation of ethylene-associated genes in transgenic parthenocarpic tomato fruits were also reported by Martinelli *et al* (2009). The accumulation of Aux/IAA transcription factor can bind to auxin responsive factor (ARF), and this interaction can activate or inhibit the transcription of auxin-responsive genes (Goetz *et al.* 2007). This study showed that the increased production of LeARF8 and LeAux/IAA may inhibit transcription of auxin-associated genes through the formation of protein complex and consequently prevent fruit set due to failure in the fertilization process.

GAs play vital role in promoting male organ formation and function, controlling size of flower, developing pollen tubes, and stimulating fruit and seed development (Olimpieri *et al.* 2011). In the present study, constitutive expression of *EgDREB1* reduced seed numbers due to deficiency in GA metabolism. It was observed that expression of *LeGA2ox2* was slightly down-regulated in both parthenocarpic lines, but remarkable down-regulated in fruits with reduced seed sets. GA2ox catalyzes 2β-hydroxylation in the GAs catabolic pathway where it is able to reduce active GAs in plants (Lo *et al.* 2008). Expression of *LeGA20ox4* was highly up-regulated in transgenic fruits. GA20ox catalyzes the oxidation of C20 GAs to produce active GAs. This finding indicates higher accumulation of GA20 in the un-pollinated ovary of all transgenic fruits. Fos *et al*. (2000) reported that GA20ox activity was higher in unpollinated ovary of *pat*-2 tomato mutant.

Involvement of cytokinin in fruit set has been reported by Matsuo *et al*. (2012) and Ding *et al*. (2013). The present study showed upregulated expression of two cytokinin-associated genes, *LeSlCKXI* and *LeSlIPT* in the transgenic tomato fruits. Adenylate isopentynyltransferase (IPT) is the first enzyme involved in CKs biosynthesis. The enzyme catalyzes production of isopentenyladenosine 5'-monophosphate (iPMP) from adenosine monophosphate (AMP) and dimethylallylpyrophosphate (DMAPP) (Takei *et al.* 2001). Cytokinin oxidase/dehydrogenase-like (CKX) is involved in inactivation of CKs by irreversible degradation. It cleaves unsaturated N6 chains from trans-zeatin, isopentynyladenine nucleotide and their corresponding ribosides (Gu *et al.* 2010, Matsuo *et al.* 2012). The up-regulation of *LeSlIPT* was higher than *LeSlCKXI* in parthenocarpic fruits as *EgDREB1* can bind to *LeSlIPT* promoter for enhancing its expression. The result also suggests that accumulation of CKs may be involved in the development of parthenocarpic fruit. However, the three transgenic lines of fruits with reduced seed set showed higher expression of *LeSlCKXI* indicating that when there was higher CKs degradation, the fruit managed to produce seeds.

The involvement of ABA in fruit development was established when the decrease in fruit size and seed numbers of tomato was observed in ABA-deficient mutants (*notabilis*/*flacca*) (Nitsch *et al.* 2012). In fruit development, ABA is also known to be involved in seed maturation and fruit ripening (Bonghi *et al.* 2011). AAO catalyzes the final reaction of ABA formation from abscisic acid aldehyde (Seo *et al.* 2000). In the present study, accumulation of ABA should have occurred when upregulation of *LeAAO* was observed in all transgenic fruits. However, there is possibility that not all *LeAAO* transcripts are converted to functional protein, thus affecting the ABA biosynthesis and reduces the availability of active ABA in the transgenic fruits. The spatial and temporal variations of mRNAs, as well as the local availability of resources for protein_biosynthesis could be a reason of the low protein abundance as reviewed by Liu *et al.* (2016).

AOS catalyzes the first step in the conversion of 13-hydroperoxy-linolenic acid to 12,13-epoxy-octadecatrienoic acid. Then, the product is converted to 12-oxo-10, 15(Z)-phytodienoic acid (OPDA). OPDA is a precursor in the biosynthesis of jasmonic acid (Ziegler *et al.* 1997, Itoh *et al.* 2002). Both jasmonate-responsive genes, *LeAOS* and *LeAOC* were up-regulated in parthenocarpic fruits. Yet, they were down-regulated in transgenic fruits with reduced seed numbers. This shows that jasmonic acids could possibly be involved in plant reproductive organ development. A similar observation was found in parthenocarpic transgenic tomato of auxin synthesis (*iaaM*) or responsiveness (*rolB*) genes driven by DefH9 or the INNER NO OUTER (INO) promoter from *Arabidopsis thaliana* (Martinelli *et al.* 2009). Taken together, our data is the first report of the gene expression that could help to explain the application of exogenous hormones such as auxin, GA, and cytokinin for parthenocarpy development. However, further studies need to be done to analyze the interaction between these phytohormone-associated genes that could help us to further understand the mechanism of fruit set without normal pollination process.

Conclusions

We obtained tomato T_0 lines showing that overexpression of *EgDREB1* gene from oil palm interfered vegetative development and seed production of their fruits. Since jasmonic acid, ethylene, auxin, cytokinin, GA, and ABA have been known to be involved in fruit development, the expression of candidate genes involved in each phytohormone biosynthetic pathway (*LeAP2*, *LeAOC*, *LeACS*, *LeACO*, *LeARF8*, *LeAux/IAA, LeSlCKX1*, *LeSlIPT1*, *LeGa2ox2*, *LeGa20ox4,* and *LeAAO*) have further elucidated the roles of these phytohormones and their possible interactions with EgDREB1. However, further study should be conducted to determine the function of *EgDREB1* at different developmental stages of transgenic fruit. Also, further analysis on the type of endogenous phytohormones and respective genes at different fruit developmental stages could explain the complex reactions involving these phytohormones and DREB1.

References

Alabadi, D., Aguero, M.S., Perez Amador, M.A., Carbonell, J.: Arginase, arginine decarboxylase, ornithine decarboxylase and polyamines in tomato ovaries-changes in unpollinated ovaries and parthenocarpic fruits induced by auxin or gibberellin. - Plant Physiol. **112**: 1237-1244, 1996.

Azzeme, A.M., Abdullah, S.N.A., Aziz, M.A., Wahab, P.E.M.: Oil palm leaves and roots differ in physiological response, antioxidant enzyme activities and expression of stress-responsive genes upon exposure to drought stress. - Acta Physiol. Plant. **38**: 1-12, 2016.

Azzeme, A.M., Abdullah, S.N.A., Aziz, M.A., Wahab, P.E.M.: 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. - Plant Physiol. Biochem. **112**: 129-151, 2017.

Bombarely, A, Menda, N., Tecle, I.Y., Buels, R.M., Strickler, S., Fischer-York, T. Pujar, A. Leto, J., Gosselin, J. Mueller, L.A.:. The Sol Genomics Network (solgenomics.net): growing tomatoes using Perl. - Nucl. Acids Res. **39** (Suppl.): D1149-D1155, 2011.

Bonghi, C., Trainotti, L., Botton, A., Tadiello, A., Rasori, A., Ziliotto, F., Zaffalon, V., Casadoro, G., Ramina, A.: A microarray approach to identify genes involved in seed-pericarp cross-talk and development in peach. - BMC Plant Biol. **11**: 1-14, 2011.

Bouaziz, D., Pirrello, J., Amor, H.B., Hammami, A., Charfeddine, M., Dhieb, A., Bouzayen M., Gargouri-Bouzid, R.: Ectopic expression of dehydration responsive element binding proteins (StDREB2) confers higher tolerance to salt stress in potato. - Plant Physiol. Biochem. **60**: 98-108, 2012.

Dhatt, A.S., Kaur, G.: Parthenocarpy: a potential trait to exploit in vegetable crops: a review. - Agr. Rev. **37**: 300-308, 2016.

Ding, J., Chen, B., Xia, X., Mao, W., Shi, K., Zhou, Y., Yu, J.: Cytokinin-induced parthenocarpic fruit development in tomato is partly dependent on enhanced gibberellin and auxin biosynthesis. - PLoS ONE **8**: 1-11, 2013.

Dos Santos, R.C., Pereira, M.C.T., Mendes, D.S., Sobral, R.R.S., Nietsche, S., Mizobutsi, G.P., Dos Santos, B.H.C.: Gibberellic acid induces parthenocarpy and increases fruit size in the 'Gefner' custard apple (*Annona cherimola Annona squamosa*). - Aust. J. Crop Sci. **10**: 314-321, 2016.

Du, L., Bao, C., Hu, T., Zhu, Q., Hu, H., He, Q., Mao, W.: SmARF8, a transcription factor involved in parthenocarpy in eggplant. - Mol. Genet. Genomics **291**: 93-105, 2016.

Ficcadenti, N., Sestili, S., Pandolfini, T., Cirillo, C., Rotino, G.L., Spena, A.: Genetic engineering of parthenocarpic fruit development in tomato. - Mol. Breed. **5**: 463-470, 1999.

Fos, M., Nuez, F., Garcia-Martin, L.: The gene *pat 2*, which induces natural parthenocarpy, alters the gibberellins content in unpollinated tomato ovaries. - Plant Physiol. **122**: 471-479, 2000.

Goetz, M., Hooper, L.C., Johnson, S.D., Rodrigues, J.C.M., Vivian-Smith, A., Koltunow, A.M.: Expression of aberrant forms of *AUXIN RESPONSE FACTOR8* stimulates parthenocarpy in *Arabidopsis* and tomato. - Plant Physiol. **145**: 351-366, 2007.

Goetz, M., Vivian-Smith, A., Johnson, S.D., Koltunow, A.M.: Auxin response factor 8 is a negative regulator of fruit initiation in *Arabidopsis*. - Plant Cell **18**: 1873-1886, 2006.

Gu, R., Fu, J., Guo, S., Duan, F., Wang, Z., Mi, G., Yuan, L.: Comparative expression and phylogenetic analysis of maize cytokinin dehydrogenase/oxidase (*CKX*) gene family. - J. Plant Growth Regul. **29**: 428-440, 2010.

Hsieh, T.H., Lee, J.T., Charng, Y.Y., Chan, M.T.: Tomato plants ectopically expressing *Arabidopsis CBF1* show enhanced resistance to water deficit stress. - Plant Physiol. **130**: 618-626 2002a.

Hsieh, T.H., Lee, J.T., Yang, P.T., Chiu, L.H., Charng, Y.Y., Wang, Y.C., Chan, M.T,: Heterology expression of the *Arabidopsis C-Repeat/Dehydration Responses Element Binding Factor 1* gene confers elevated tolerance to chilling and oxidative stresses in transgenic tomato. - Plant Physiol. **129**: 1086-1094, 2002b.

Ito, Y., Katsura, K., Maruyama, K., Taji, T., Kobayashi, M., Seki, M., Shinozaki, K., Yamaguchi-Shinozaki, K.: Functional analysis of rice DREB1/CBF-type transcription factors involved in cold-responsive gene expression in transgenic rice. - Plant Cell Physiol. **47**: 141-153, 2006.

Itoh, A., Schilmiller, A.L., McCaig, B.C., Howe, G.A.: Identification of a jasmonate-regulated allene oxide synthase that metabolizes 9-hydroperoxides of linoleic and linolenic acids. - J. biol. Chem. **277**: 46051-46058, 2002.

Kasuga, M., Liu, Q., Miura, S., Yamaguchi-Shinozaki, K., Shinozaki, K.: Improving plant drought, salt, and freezing tolerance by gene transfer of a single stress-inducible transcription factor. - Natur. Biotechnol. **17**: 287-291, 1999.

Koshioka, M., Nishijima, T., Yamazaki, H., Liu, Y., Nonaka, M., Mander, L.N.: Analysis of gibberellins in growing fruits of *Lycopersicon esculentum* after pollination or treatment with 4-chlorophenoxyacetic acid. - J. hort. Sci. **69**: 171-179, 1994.

Li, S., Zhao, Q., Zhu, D., Yu, J.: A DREB transcription factor from maize (*Zea mays*), ZmDREB4.1 plays a negative role in plant growth and development. - Front. Plant Sci. **9**: 1-15, 2018.

Li, X.X., Kobayashi, F., Ikeura, H., Hayata, Y.: Chlorophenoxyacetic acid and chloropyridylphenylurea accelerate translocation of photoassimilates to parthenocarpic and seeded fruits of muskmelon (*Cucumis melo*). - J. Plant Physiol. **168**: 920-926, 2011.

Liu, L., Wang, Z., Liu, J., Liu, F., Zhai, R., Zhu, C., Wang, H., Ma, F., Xu, L.: Histological, hormonal and transcriptomic reveal the changes upon gibberellin-induced parthenocarpy in pear fruit. - Hort. Res. **5**: 1-13, 2018.

Liu, Y., Beyer, A., Aebersold, R.: On the dependency of cellular protein levels on mRNA abundance. - Cell **165**: 535-550, 2016.

Lo, S.F., Yang, S.Y., Chen, K.T., Hsing, Y.I., Zeevaart, J.A.D., Chen, L.J., Yu, S.M.: A novel class of gibberellin 2-oxidases control semidwarfism, tillering, and root development in rice. - Plant Cell. **20**: 2603-2618, 2008.

Martinelli, F., Uratsu, S.L., Reagan, R.L., Chen, Y., Tricoli, D., Fiehn, O., Rocke, D.M., Gasser, C.S., Dandekar, A.M.: Gene regulation in parthenocarpic tomato fruit. - J. exp. Bot. **60**: 3873-3890, 2009.

Martinez, C., Manzano, S., Megias, Z., Garrido, D., Pico, B., Jamilena, M.: Involvement of ethylene biosynthesis and signalling in fruit set and early fruit development in zucchini squash (*Cucurbita pepo* L.). - BMC Plant Biol. **13**: 1-14, 2013.

Matsuo, S., Kikuchi, K., Fukuda, M., Honda, I., Imanis, S.: Roles and regulation of cytokinins in tomato fruit development. - J. exp. Bot. **63**: 5569-5579, 2012.

Morran, S., Eini, O., Pyvovarenko, T., Parent, B., Singh, R., Ismagul, A., Eliby, S., Shirley, N., Langridge, P., Lopato, S.: Improvement of stress tolerance of wheat and barley by modulation of expression of DREB⁄CBF factors. - Plant Biotechnol. J. **9**: 230-249, 2011.

Nishawy, E., Sun, X., Ewas, M., Ziaf, K., Xu, R., Wang, D., Amar, M., Zeng, Y., Cheng, Y.: Overexpression of *Citrus grandis DREB* gene in tomato affects fruit size and accumulation of primary metabolites. - Sci. Hort. **192**: 460-467, 2015.

Nitsch, L., Kohlen, W., Oplaat, C., Charnikhova, T., Cristescu, S., Michieli, P., Wolters-Arts, M., Bouwmeester, H., Mariani, C., Vriezen, W.H., Rieu I.: ABA-deficiency results in reduced plant and fruit size in tomato. - J. Plant Physiol. **169**: 878-883, 2012.

Olimpieri, I., Caccia, R., Picarella, M.E., Pucci, A., Santangelo, E., Soressi, G.P., Mazzucato A.: Constitutive co-suppression of the *GA 20-oxidase1* gene in tomato leads to severe defects in vegetative and reproductive development. - Plant Sci. **180**: 496-503, 2011.

Osborne, D.J., Went, F.W.: Climatic factors influencing parthenocarpy and normal fruit-set in tomatoes. - Int. J. Plant Sci. **114**: 312-322, 1953.

Pasaresi, P., Mizzotti, C., Colombo, M.. Masiero, S.: Genetic regulation and structural changes during tomato fruit development and ripening. - Front. Plant Sci. **5**: 1-14, 2014.

Qian, C., Ren, N., Wang, J., Xu, Q., Chen, X., Qi, X.: Effects of exogenous application of CPPU, NAA and GA4+7 on parthenocarpy and fruit quality in cucumber (*Cucumis sativus* L.). - Food Chem. **243**: 410-413, 2018.

Ramin, A.A.: Effects of auxin application on fruit formation in tomato growing under stress temperatures in the field. - J. hort. Sci. Biotechnol. **78**: 706-710, 2003.

Rejeb, K. B., Abdelly, C., Savoure, A.: How reactive oxygen species and proline face stress together. - Plant Physiol. Biochem. **80**: 278-284, 2014.

Rasul, M.G., Mian, M.A.K., Cho. Y., Ozaki, Y., Okubo, H.: Application of plant growth regulators on the parthenocarpic fruit development in teasle gourd (Kakrol, *Momordica dioica* Roxb.). - J. Fac. Agr. Kyushu Univ. *53*: 39-42, 2008.

Rojas-Gracia, P., Roque, E., Medina, M., Rochina, M., Hamza, R., Angarita-Diaz, M.P., Moreno, V., Pérez-Martín, F., Lozano, R., Cañas, L., Beltrán, J.P., Gómez-Mena, C.: The parthenocarpic hydra mutant reveals a new function for a *SPOROCYTELESS*-like gene in the control of fruit set in tomato. - New Phytol. **214**: 1198-1212, 2017.

Seo, M., Peeters, A.J.M., Koiwai, H., Oritani, T., Marion-Poll, A., Zeevaart, J.A.D., Koornneef, M., Kamiya, Y., Koshiba, T.: The *Arabidopsis* aldehyde oxidase 3 (*AAO3*) gene product catalyzes the final step in abscisic acid biosynthesis in leaves. - Proc. nat. Acad. Sci. USA **97**: 12908-12913, 2000.

Soltesz, A., Smedley, M., Vashegyi, I., Galiba, G., Harwood, W., Vagujfalvi, A.: Transgenic barley lines prove the involvement of *TaCBF14* and *TaCBF15* in the cold acclimation process and in frost tolerance. - J. exp. Bot. 64: 1849-1862, 2013.

Stam, M., Mol, J.N.M., Kooter, J.M.: The silence of genes in transgenic plants. - Ann. Bot. **79**: 3-12, 1997.

Serrani, J.C., Fos, M., Atares, A., Garcia-Martinez, J.L.: Effect of gibberellin and auxin on parthenocarpic fruit growth induction in the cv. Micro-tom of tomato. - J. Plant Growth Regul. **26**: 211-221, 2007.

Takei, K., Sakakibara, H., Sugiyama, T.: Identification of genes encoding adenylate isopentenyltransferase, a cytokinin biosynthesis enzyme, in *Arabidopsis thaliana*. - J. biol. Chem. **276**: 26405-26410, 2001.

Takisawa, R., Nakazaki, T., Nunome, T., Fukuoka, H., Kataoka, K., Saito, H., Habu, T., Kitajima, A.: The parthenocarpic gene *Pat-k* is generated by a natural mutation of *SlAGL6* affecting fruit development in tomato (*Solanum lycopersicum* L.). - BMC Plant Biol. **18**: 1-12, 2018.

Tu, D., Luo, Z., Wu, B., Ma, X., Shi, H., Mo, C., Huang J., Xie, W.: Developmental, chemical and transcriptional characteristics of artificially pollinated and hormone-induced parthenocarpic fruits of *Siraitia grosvenorii*. - Roy. Soc. Chem. Adv. **7**: 12419-12428, 2017.

Ueta, R., Abe, C., Watanabe, T., Sugano, S.S., Ishihara, R., Ezura. H., Osakabe, Y., Osakabe, K.: Rapid breeding of parthenocarpic tomato plants using *CRISPR/Cas9*. - Sci. Rep. **7**: 1-8, 2017.

Wang, H., Jones, B., Li, Z., Frasse, P., Delalande, C., Regad, F., Chaabouni, S., Latché, A. Pech, J.C., Bouzayen, M.: The tomato Aux/IAA transcription factor IAA9 is involved in fruit development and leaf morphogenesis. - Plant Cell. **17**: 2676-2692, 2005.

Wang, J.W., Yang, F.P., Chen, X.Q., Liang, R.Q., Zhang, L.Q., Geng, D.M., Zhang, X.D., Song, Y.Z., Zhang, G.S.: Induced expression of *DREB* transcriptional factor and study on its physiological effects of drought tolerance in transgenic wheat. - Acta genet. sin. **33**: 468-476, 2006.

Zhang, P., Yang, P., Zhang, Z., Han, B., Wang, W., Wang, Y., Cao, Y., Hu, T.: Isolation and characterization of a buffalograss (*Buchloe dactyloides*) dehydration responsive element binding transcription factor, *BdDREB2*. - Gene **536**: 123-128, 2014.

Ziegler, J., Hamberg, M., Miersch, O., Parthier, B,: Purification and characterization of allene oxide cyclase from dry corn seeds. - Plant Physiol. **114**: 565-573, 1997.