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 plants, 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-carboxylic acid 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 transgenic plants by regulating the expression of hormone-associated genes.

Additional key words: abscisic acid, auxin, cytokinin, ethylene, 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...
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 GA3 or GA4 can induce parthenocarpy in pear, but the use of GA3 did not produce parthenocarpic trait in pear fruit. However, application of GA3 produced parthenocarpic grape, tomato, custard apple (Dos Santos 2016), and Siraitia grosvenorii (Tu et al. 2017). The application of cytokinin like compound (N-(2-chloro-4-pyridyl)-N-phenylurea; CPPU) induced parthenocarpic tease 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 Severinian 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-linc1 tomatoes are controlled by the recessive genes pat3/pat4, pat4.1/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 parthenocarpic 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-dependent 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 results 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 (Soltész 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 adenylyl isopentenyltransferase. These genes contain DRE/CRT motif in their promoter sequence (Azzeme et al. 2017). Overexpression of citrus DREB1 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. MTI) seeds were purchased from the Malaysian Agricultural Research and Development Institute (MARDI), Serdang and kept at 4 °C. The seeds were further germinated on Murashige 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 (Qiaogen, Hilden, Germany). The putative transgenic plants with pMDC(-32) were verified by PCR with CaMV 35S promoter primers (forward primer: 5'-ATTCTTTGCCCCCTCGGAGTCT-3'; reverse primer: 5'-TTAAAGTGATTGTCCTGAGTCT-3'), while transgenic lines with EgDREB1 were verified by hygromycin resistance gene (hptII) primers (forward primer: 5'-ATTCTTTTGCCCCCTCGGAGTCT-3'; reverse primer: 5'-AAAGCTTGAATCTACCGCGAGCT-3').

Six independent transgenic lines harboring Elaeis guineensis DREB1 (EgDREB1) (named as EgDREB1-L5, EgDREB1-L9, EgDREB1-L12, EgDREB1-L5, EgDREB1-L3, and EgDREB1-L10), transgenic plant harboring empty vector [named as pMDC(-32)] and WT
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), aminocyclopentane-1-carboxylic acid synthase (LeACS), 1-aminocyclopropane-1-carboxylate oxidase 1 (LeACO), auxin responsive factor 8 (LeARF8), auxin/indole-3-acetic acid (LeIaa/IAA), cytokinin oxidase/dehydrogenase-like (LeSCKX1), adenylate isopentenyltransferase (LeSPTI), 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/) (Bomareley 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 fruits and flowers 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 °C ± 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 °C. 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>
<thead>
<tr>
<th>Plant type</th>
<th>Number of seeds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>720</td>
</tr>
<tr>
<td>pMDC(-32)</td>
<td>700</td>
</tr>
<tr>
<td>EgDREB1-L5</td>
<td>0</td>
</tr>
<tr>
<td>EgDREB1-L9</td>
<td>0</td>
</tr>
<tr>
<td>EgDREB1-L1</td>
<td>33</td>
</tr>
<tr>
<td>EgDREB1-L3</td>
<td>44</td>
</tr>
<tr>
<td>pMDC-EgDREB1-L10</td>
<td>39</td>
</tr>
<tr>
<td>pMDC-EgDREB1-L13</td>
<td>30</td>
</tr>
</tbody>
</table>
gene expression analysis was carried out in fruits. The calibrator used to analyze the expression of 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, LeGA2ox4), 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. 3A). 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. 3B) and LeACO (Fig. 3C) 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. 3D and Fig. 3E, 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
was down-regulated, K - (having the highest Ct value), which was set to 1.

in the G;

was up-regulated by 2

is involved in regulation of expression of

4603 (+), 4603 (-) = 3. Fold

1

2 (Fig. 3K) was

and EgDREB1-L13 (0.46-fold) compared to the WT.

EgDREB1-L3 (0.03-fold), EgDREB1-L10 (0.12-fold),

EgDREB1-L9 (0.84-fold), EgDREB1-L1 (0.05-fold),

EgDREB1-L13 (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. 3L). 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 LeSIPT1 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

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; LeSIPT1 - adenylate isopentenyltransferase; (+) - sense strand; (-) - antisense strand.

<table>
<thead>
<tr>
<th>Name of gene</th>
<th>Copy number</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>LeARF8</td>
<td>2</td>
<td>4603 (+), 4603 (-)</td>
</tr>
<tr>
<td>LeAP2</td>
<td>2</td>
<td>800 (+), 1602 (-)</td>
</tr>
<tr>
<td>LeACS</td>
<td>2</td>
<td>4946 (+), 4946 (-)</td>
</tr>
<tr>
<td>LeGA2ox2</td>
<td>2</td>
<td>3 (+), 4580 (+)</td>
</tr>
<tr>
<td>LeSIPT1</td>
<td>1</td>
<td>2080 (-)</td>
</tr>
</tbody>
</table>
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,
- 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 °C and 28 - 30 °C, respectively. The dwarf 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, LeSICXI and LeSIPT in the transgenic tomato fruits. Adenylate isopentenyltransferase (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, isopentyladenylate nucleotide and their corresponding ribosides (Gu et al. 2010, Matsuo et al. 2012). The up-regulation of LeSIPT was higher than LeSICX1 in parthenocarpic fruits as EgDREB1 can bind to LeSIPT 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 LeSICX1 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 (See 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 (rollB) genes driven by DeffH9 or the INNER NO OUTER (INO) promoter from Arabidopsis thaliana (Martellini 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 parthenocarpic 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 Tn 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, LeSICX1, LeSIPT1, 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


