Scientia Horticulturae 280 (2021) 109940

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Effects of temperature, soil moisture and light intensity on the temporal pattern of floral gene expression and flowering of avocado buds (*Persea americana* cv. Hass)

Aleyda Acosta-Rangel *, Rui Li, Peggy Mauk, Louis Santiago, Carol J. Lovatt

Department of Botany & Plant Sciences, University of California, Riverside, CA, 92521-0124, USA

ARTICLE INFO

Keywords:
AGAMOUS
APETALA 1, *2* and *3*
Floral meristem determinacy
FLOWERING LOCUS T
LEAFY
PISTILLATA

ABSTRACT

For 'Hass' avocado (*Persea americana* Mill.), low temperature is known to induce flowering, but effects of other environmental factors remain equivocal. In addition, documentation of interactions among environmental factors, floral gene expression and subsequent flower development is limited. Thus, in this research, the effects of environmental factors on the temporal expression patterns of genes related to flowering time, floral meristem identity and floral organ identity were quantified in buds relative to inflorescence number. 'Hass' avocado trees were subjected to four different environmental conditions: optimal growth condition (OGC) (warm temperature, well-watered, photosynthetic active radiation > 900 μmoles m^{-2} s^{-1}), low temperature (LT), low soil moisture (LSM) or low light intensity (LLI) for 8 weeks, followed by OGC for 6 weeks. Bud expression of homologs of *Arabidopsis thaliana* flowering genes *FLOWERING LOCUS T* (*FT*), *LEAFY* (*LFY*), *APETALA1* (*AP1*), *APETALA2* (*AP2*), *APETALA3* (*AP3*), *PISTILLATA.1* (*PI.1*), *AGAMOUS.1* (*AG.1*) and *AGAMOUS.3* (*AG.3*) were quantified over time. Only buds of LT-treated trees flowered. At the start of the experiment (week 0), buds of all trees expressed *PaLFY*, *PaAP1* and *PaAP2*. After 4 weeks of treatment, bud *PaAP1* expression was greater than in week 0 and continued to increase through week 8 only in LT-treated trees, whereas by week 8 *PaLFY* expression increased in LT- and LSM-treated trees. By week 10 (2 weeks after transfer to OGC), bud *PaFT* expression increased only in LT-treated trees, followed by activation of downstream floral organ identity genes *PaAP3*, *PaPI.1*, *PaAG.1* and *PaAG.3* by week 12. For OGC-, LSM- and LLI-treated trees, bud *PaAP1*, *PaFT*, *PaAP3*, *PaPI.1* and *PaAG.1* expression remained low, with *PaAG.3* transcripts undetected at week 12. These trees did not flower. Among environmental factors tested, only LT increased bud expression of both *PaLFY* and *PaAP1*, which likely conferred floral meristem determinacy since transfer of LT-treated trees to OGC did not prevent flowering but activated *PaFT*, downstream floral organ identity genes, and flowering. The results suggest 'Hass' avocado floral development is promoted by low temperature-dependent floral induction and warm temperature-dependent floral organogenesis.

1. Introduction

In an era of climate change, it is important to identify environmental factors that promote or prevent flowering of commercial crops. Environmental factors documented to induce flowering across species include, but are not limited to, low and high temperature, low and high soil moisture (i.e., water deficit and hypoxia, respectively), low and high light intensity, UV light, and nutrient deficiency (Takeno, 2016). Among tropical and subtropical tree crops, low temperature and water deficit are both known to induce flowering in *Citrus* spp. (Southwick and Davenport, 1986; Lovatt et al., 1988; Nakajima et al., 1992), mango (*Mangifera indica*) (Núñez-Elisea and Davenport, 1994), and litchi (*Litchi chinensis*) (Stern et al., 1993; Shen et al., 2016). In contrast, low temperature, but not low soil moisture, results in flowering in longan (*Dimocarpus longan*) (Chen et al., 2010), whereas only water deficit promotes significant flowering in star fruit (*Averrhoa carambola*) (Salakpetch et al., 1990). Although low temperature is well known to induce flowering in *Persea americana*, cv. Hass and Fuerte (Mexican x Guatemalan hybrids) (Buttrose and Alexander, 1978; Nevin and Lovatt, 1989; Chaikiattiyos et al., 1994; Salazar-García et al., 1999), effects of other environmental factors on flowering of 'Hass'avocado, the cultivar that dominates the global avocado industry, are less clear. Low temperature

* Corresponding author at: University of Florida, 14625 CR672, Wimauma, FL, 33598, USA.
E-mail address: aacos011@ucr.edu (A. Acosta-Rangel).

https://doi.org/10.1016/j.scienta.2021.109940
Received 7 July 2020; Received in revised form 18 December 2020; Accepted 23 December 2020
Available online 23 January 2021

with low light intensity was observed to delay and concentrate 'Hass' avocado flowering to warm sunny days (Buzgo et al., 2007). However, reducing light intensity 50 % in combination with low temperature did not significantly affect flower number compared to low temperature alone (Buttrose and Alexander, 1978). In an experiment testing the effect of reduced soil moisture content on avocado flowering, water deficit stopped vegetative shoot extension growth, a putative prerequisite for the transition of the shoot apical meristem (SAM) from vegetative to reproductive development, but failed to promote flowering (Chaikiattiyos et al., 1994). Given the limited number of reports in the literature investigating the effects of environmental factors on avocado flowering, it is clear that further research is required to understand and potentially mitigate negative effects of the environment and global climate change on avocado floral development and productivity.

For 'Hass' avocado trees, transition from the vegetative to reproductive SAM was documented to occur at the end of the summer flush of vegetative shoot (extension) growth, based on anatomical changes in the primary axis meristem and formation of two lateral secondary axis inflorescence meristems (Salazar-García et al., 1998; Buzgo et al., 2007). However, floral development was not destined at this point, but dependent on environmental conditions that promoted flowering and development of additional secondary axis meristems that prevented further vegetative growth. Buds were committed to floral development when three or more secondary axis inflorescence meristems had developed (Salazar-García et al., 1999). Using this criterion, irreversible commitment of the terminal bud to floral development was documented to occur from the end of October through November in California and Mexico (Salazar-García et al., 1998, 2006), but slightly later in Florida (Buzgo et al., 2007) and Israel (Ziv et al., 2014), suggesting that low temperatures of autumn into winter might regulate floral induction.

In *Arabidopsis thaliana*, and many other plant species, *FLOWERING LOCUS T* (*FT*) and *LEAFY* (*LFY*) are floral timing genes (Moon et al., 2005; Parcy, 2005; Lee and Lee, 2010). *LFY* also functions as a floral meristem identity gene with *APETALA1* (*AP1*) (Blazquez et al., 2006; Benlloch et al., 2007; Sablowski, 2007; Hong and Jackson, 2015; Ma, 1994). The expression patterns of *PaFT, PaLFY* and *PaAP1* genes in leaves and buds of 'Hass' avocado trees growing in Israel suggested that low temperatures in fall increased *PaFT* expression in leaves, which subsequently upregulated *PaAP1* and *PaLFY* expression in buds and resulted in the formation of two secondary axis inflorescence meristems by the end of November (Ziv et al., 2014). The results are consistent with the roles *FT*, *LFY* and *AP1* in floral initiation in *A. thaliana* (Melzer et al., 1999).

As a member of the Lauraceae, *P. americana* is a basal angiosperm (noncore eudicot). The flower of *P. americana* is characteristic of basal angiosperms, with the first and second whorls comprising an undifferentiated perianth of similar petaloid tepals and multiple whorls of stamens, including staminodes, which surround a single carpel (Blanke and Lovatt, 1992; Chanderbali et al., 2006, 2009). The expression patterns of the genes that specify floral organ development in *P. americana* are broad and overlapping compared to the specification of *A. thaliana* floral organ identity described by the ABC model (Chanderbali et al., 2006, 2009, 2010, 2016). In the ABC model, A function genes *AP1* and *APETALA2* (*AP2*) specify sepals, A function genes plus the B function genes *APETALA3* (*AP3*) and *PISTILLATA* (*PI*) specify petals, B function genes plus the C function gene *AGAMOUS* (*AG*) (antagonistic to the A-function genes) specify stamens, and the C function gene alone specifies the carpel (Bowman et al., 1991; Coen and Meyerowitz, 1991; Krizek and Fletcher, 2005). In the *P. americana* flower, *PaAP1* is expressed in the whorls of tepals and stamens, with the homologs *PaAP3* and *PaPI.1* (putative B function genes) also expressed in the whorls of tepals and the stamens (Chanderbali et al., 2006, 2009; Soltis et al., 2007a, b). Three *PaAG* homologs (putative C function genes) were identified in *P. americana*, with *PaAG.1* and *PaAG.2* expressed in outer and inner tepals, stamens and carpel, and *PaAG.3* expression restricted to stamens and carpel (Chanderbali et al., 2006, 2009).

Despite the very thorough analysis of homologs of *A. thaliana* floral organ identity genes expressed in the avocado flower (Chanderbali et al., 2006, 2008, 2009; Soltis et al., 2007a, b, 2009), it is a striking omission that the expression of these genes has not yet been integrated with expression patterns of classic upstream genes having floral timing (promoter, integrator) function, *FT* and *LFY* (Moon et al., 2005; Parcy, 2005; Lee and Lee, 2010), or with genes that function in floral meristem identity, *LFY* and *AP1* (Bowman et al., 1991; Ratcliffe et al., 1999; Parcy, 2005; Benlloch et al., 2007; Siriwardana and Lamb, 2012; Ma, 1994) and that no data have been published documenting the effects of environmental factors on the expression of floral organ identity genes in relation to inflorescence development and flower formation. Further, it should be noted that the proposed roles for *PaLFY* and *PaAP1* in floral meristem determinay in buds of the 'Hass' avocado (Ziv et al., 2014) have not been confirmed. In addition, a possible second function of *PaFT* in flower developmental processes in the avocado bud prior to anthesis proposed by Ziv et al. (2014) requires further investigation. Low and warm temperature treatments provide valuable tools to promote and prevent flowering of 'Hass' avocado, respectively, for comparative analysis of floral gene expression to increase our knowledge of the roles played by these genes in floral induction and achieving floral meristem determinacy and ultimately flower formation in 'Hass' avocado buds. Understanding the regulation of these processes in response to different environmental factors is essential for improving crop yield (Hong and Jackson, 2015).

Thus, the research presented herein was undertaken to compare the temporal patterns of expression of genes putatively regulating floral timing (induction) (*PaFT* and *PaLFY*), floral meristem identity (determinacy) (*PaLFY* and *PaAP1*), and floral organ identity (*PaAP2*, *PaAP3, PaPI.1, PaAG.1* and *PaAG.3*) (flower formation) in buds of 'Hass' avocado trees under (1) low temperature conditions known to promote significant flowering and (2) optimal growing conditions (warm temperature, well-watered, photosynthetic active radiation $>$ 900 μmoles m^{-2} s^{-1}) known to sustain vegetative shoot growth. Using the low temperature treatment as the positive control, a second objective was to quantify the effects of two additional environmental factors, low soil moisture content and low light intensity, on bud floral gene expression relative to the developmental fate of avocado buds. The results reported herein are the first to quantify the effects of four different environmental conditions on bud expression of flowering-related genes and inflorescence number of 'Hass' avocado trees. The results also provide the first evidence demonstrating the relationship between *PaLFY* and *PaAP1* transcript levels in avocado and upregulation of *PaFT* and floral organ identity genes when flower formation was successful and the failure of specific genes to be activated under environmental conditions that did not promote flowering.

2. Materials and methods

2.1. Plant material and treatment conditions

Adult 'Hass' avocado trees (3.5 years from budding onto clonal Duke 7 rootstocks), grown in 12-L plastic tubes containing steam-sterilized potting soil, were obtained from Brokaw Nursery, Ventura, California. All flowers and fruit were removed to prevent adverse effects on floral gene expression and flowering (Ziv et al., 2014). For the 5 months prior to the initiation of the experiment, the trees were maintained under optimal growth conditions (OGC) in a glasshouse (14-hr day, using supplemental lighting, with photosynthetic active radiation [PAR] $>$ 900 μmoles m^{-2} s^{-1} at 30 °C/10-hr night at 20 °C; humidity averaged 80 %). Trees were irrigated with 1.2 L of water per day to maintain a soil volumetric water content (VWC) between 20 %–25 %. The experiment was initiated on 15 July during the summer flush of vegetative shoot extension growth, at which time terminal buds were vegetative. Trees were randomly assigned to one of four treatments: (1) optimal growth conditions (OGC, negative control), described above; (2) low

temperature (LT, positive control), OGC modified to a 10-hr day at 14 °C/14-hr night at 10 °C, using a Percival PGW growth chamber (2.3 × 1.5 × 2.0 m; Percival, Boone, IA); (3) low soil moisture (LSM), OGC with soil VWC maintained between 8 %–12 % by deficit irrigation (0.6 L of water every 3 days); and (4) low light intensity (LLI), OGC with PAR < 130 μmoles m^{-2} s^{-1} using black net shade cloth to reduce light intensity 85 % (Fig. 1). After 8 weeks of treatment, all trees were transferred to OGC in a glasshouse. During the 14-week experiment, all trees were fertilized equally with half-strength Hoagland's nutrient solution (Hoagland and Arnon, 1950). Data loggers (Campbell Scientific CR1000, Logan, UT) were used to monitor environmental conditions. To determine treatment effects on tree water status, midday leaf water potential (Ψ_{midday}) was measured every 2 weeks with a pressure chamber (1001, PMS Instruments, Albany, OR).

For each treatment, at weeks 4, 8, 10 and 12, the distal five buds from 10 nonbearing shoots were collected from three individual trees (replications) per date, for a total of 12 trees per treatment. At the start of the experiment (week 0), samples were collected from all trees, using four trees per sample to create three biological replications. In all cases, collected samples were placed between moistened paper towels inserted into insulated bags inside labeled plastic bags, which were sealed, placed in a cooler box and transported to the laboratory (< 5 min). In the laboratory, the shoots comprised of 5 distal buds, with leaves removed, were immediately frozen in liquid nitrogen and stored at −80 °C until used for RNA extraction. Total RNA was extracted from bud tissue, previously ground in liquid nitrogen, using Isolate Plant RNA Mini Kit (Bioline USA Inc., Taunton, MA). The RNA quality and quantity were analyzed by spectroscopy using a Nanodrop 2000 spectrophotometer (Thermo Scientific, Wilmington, DE) and an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Carla, CA). For cDNA synthesis, 1 μg of total RNA was treated with RQ1 RNase-Free DNase (Promega, Madison, WI) to eliminate any DNA contamination. First-strand cDNA synthesis was performed using a Tetro cDNA Synthesis Kit (Bioline USA Inc., Taunton, MA) with oligo (dT) primer in a 30 μL reaction at 42 °C for 60 min according to the protocol of the manufacturer.

2.2. PCR primer design and amplification efficiency

The sequences of *A. thaliana* homologs *FT*, *LFY*, *AP1*, *AP2*, *AP3*, *PI*, *AG* and *β-ACT* in *P. americana* were obtained from the reference sequence database of the National Center for Biotechnology Information (NCBI, 2018), except *P. borbonia AP2*, which was obtained from the 1000 Green Plant Transcriptome Project, University of Alberta, Canada (Matasci et al., 2014) (Table 1).

Primers for *P. americana FT* and *LFY* used in this research were those of Ziv et al. (2014) and Chanderbali (unpublished personal communication), respectively. The seven additional primer sets were designed using the website PrimerQuest Tool from Integrated DNA Technologies Company (Integrated DNA Technologies (IDT, 2018). For primer design, the following filters were used: melting temperatures (Tm) of 60–62 °C, primer lengths of 18–24 bp, and amplicon lengths of 150–297 bp. The annealing temperature and concentration of the primer sets were optimized for Quantitative Real-time PCR (qPCR) to efficiencies within the range of 87%–100 %. The size and sequence of the amplicon products were verified by 2 % (w/v) gel electrophoresis and sequence analysis provided by the Institute for Integrative Genome Biology, University of California, Riverside, and each compared with its respective target gene sequence in *P. americana* using BLAST (NCBI web page) and ClustalW (Geneious Software, version 10.2.3) (Table 1).

2.3. Quantitative real-time PCR analysis

Quantitative real-time PCR was carried out using a C1000 Touch™ thermal cycler (Bio-rad Laboratories, Hercules, CA) with the CFX96 Touch™ real-time PCR detection system. The final reaction volume was 18 μL containing 100 ng of cDNA in 2 μL, 0.6 μL of gene-specific forward and reverse primer mix (10 nM), 9 μL of SensiMix™ SYBR & Fluorescein (2X) mix (Bioline USA Inc., Taunton, MA), and 6.4 μL of PCR-grade water. Each reaction was run at 95 °C for 10 min, followed by 40 cycles of 95 °C for 10 s and 60 °C for 1 min. Melt-curve analysis ranging from 60 to 95 °C was performed at the end of each qPCR run to confirm that nonspecific products were not formed. Only quantification cycle (Cq) values less than 35 were used to calculate the relative expression levels (fold change) of the target genes using the Pfaffl method (Pfaffl, 2001) with 'Hass' avocado flowers collected from orchard trees at full bloom as the control (expression level of 1) and *β-Actin* (*PaACT*) (Table 1) as the reference gene (endogenous control). Relative expression values reported herein for *PaFT*, *PaAP3*, *PaPI.1*, *PaAG.1* and *PaAG.3* are low due to the significant expression of these genes in the avocado flowers used as the control (expression level of 1); average Cq values for these genes averaged between 23.8 and 31.0. Gene expression data for each treatment were the means of three biological replications; each biological replication was the mean of three qPCR technical replications.

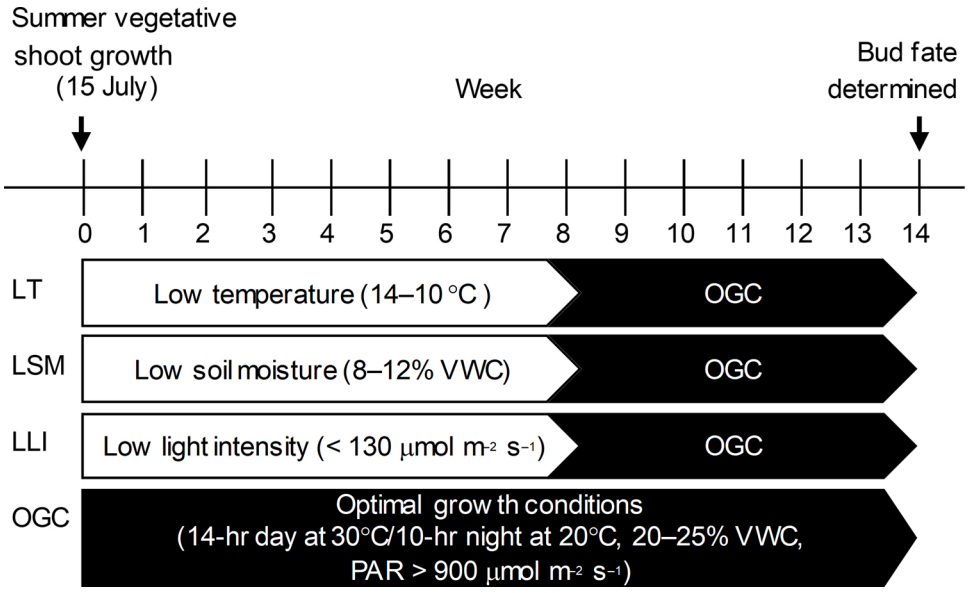

Fig. 1. At week 0, 'Hass' avocado trees were subjected to one of four environmental conditions for 8 weeks: (1) optimal growth conditions (OGC), 14-hr day at 30 °C, with photosynthetic active radiation (PAR) > 900 μmoles m^{-2} s^{-1} /10-hr night at 20 °C; soil volumetric water (VWC) between 20 % to 25 %; relative humidity ~80 % (2) low temperature (LT, positive control), OGC modified to a 10-hr day at 14 °C/14-hr night at 10 °C; (3) low soil moisture (LSM), OGC with soil VWC maintained between 8 % to 12 %; and (4) low light intensity (LLI), OGC with PAR < 130 μmoles m^{-2} s^{-1}. At the end of 8 weeks of treatment, all trees were grown under OGC for an additional 6 weeks.

Table 1
Forward and reverse primers for the target floral genes and *β-ACT* for *Persea americana* used in the quantitative real-time PCR (qPCR) assays.

Annotation	Accession number* (*Species*)	Forward primer (5' to 3') Reverse primer (5' to 3')	Product size (bp)	PCR product sequence blasted against target gene sequence	
				E-value	Identity
PaFT	KM023154.1 (*P. americana*)	TCCGGGGTGGCGTCAGAACT TCTCCGGCTGTCGTCGGACT	142	5E-50	98 %
PaLFY	FD502004.1 (*P. americana*)	GCAGCGTGAACATCCCTTCATTGT TGGATCAAGAACTCCCTGCACTGT	114	5E-60	100 %
PaAP1	DQ398019.1 (*P. americana*)	CATTCACCATCCTTGCTACTG GAGCACCTACTTCCTCTTCT	105	9E-21	100 %
PaAP2	WIGA-2009052 (*P. americana*)	GGCCCAAGTAGACGTATTTC TCGACAAAGTACCGGATTTC	122	5E-27	97 %
PaAP3	AY337748.1 (*P. americana*)	TGCGAGCATTGGAAGGAA GCATGGTTGGATGCAGAAAG	130	1E-13	90 %
PaPI.1	AY337738.1 (*P. americana*)	CAGATGGAGTTCTTAAGGGCACTC GATATTTGCTGCTGATGCAA	88	4E-38	99 %
PaAG.1	DQ398021.1 (*P. americana*)	AGAACGCAAACAGGCATCTG CTACTGATGCCTTTCTCCAATCT	98	1E-13	87 %
PaAG.3	DQ398023.1 (*P. americana*)	GCACTCCAGCTAGGATGATAAA CTAGGAACTGCAGCCTTCAA	109	4E-13	95 %
PaACT	GU272027.1 (*P. americana*)	AACATTGTGCTTAGCGGTGGTTCC TCCACATCTGTTGGAAGGTGCTCA	183	3E-78	96 %

* The database sources for the accession numbers: NCBI GenBank and Reference Sequence databases (http://www.ncbi.nlm.nih.gov) (NCBI, 2018), and 100 Plants (www.onekp.com/) (Matasci et al., 2014).

2.4. Treatment effects on bud development

The fate of the distal five buds from six (nonbearing) shoots per tree (30 buds/tree) for each of six individual trees (replications) for each of the four treatments was determined. This included identifying the developmental stage of each bud according to the visual classification scale of Salazar-García et al. (1998) and quantifying the number of buds at each stage at weeks 0, 12 and 14. No shoots were collected from these trees to prevent changes in the fate of the bud. The fate of the five distal buds (1 terminal bud and 4 axillary buds) on six shoots per tree were averaged for the six individual trees (replications) per treatment and reported as the average value for 30 buds per tree. The developmental stage of terminal and axillary buds was photographed at weeks 0, 4, 8 and 12.

2.5. Statistical analysis

All statistical analysis was performed using R software version 3.4.3 (R Core Team, 2013). All data were tested for linear model assumptions using Kolmogorov–Smirnov and Levene tests. For variables related to bud fate, a general linear model with Poisson correction was used to determine the effect of treatments for a given week on the stage of bud development and the number of floral shoots, vegetative shoots and inactive (quiescent) buds per tree. Significant differences were considered with a family error rate $\alpha \leq 0.05$. Post-hoc comparisons were performed using a pairwise *t*-test with Bonferroni adjustment. Relative expression data were transformed using $\log_{10}$ function in order to obtain a symmetrical distribution. Analysis of variance (ANOVA) was used to compare the effect of treatments within a week and over time (across weeks) for a given treatment. When ANOVA testing indicated significant differences at $\alpha \leq 0.05$ for equal variances, Duncan's multiple range test was performed to identify differences between treatments and weeks, respectively. When ANOVA testing indicated significant differences at $\alpha \leq 0.01$ for unequal variances, the non-parametric Kruskal-Wallis test was used to identify differences between treatments and weeks, respectively. Data were back-transformed for presentation in Figs. 4 and 5. When the expression level of the target gene in each of the three biological replications was below the threshold value for detection (quantification cycle (Cq) in qPCR ≥ 35), the results were reported as not detected (ND) (Fig. 5). Pearson's product-moment correlation coefficients were calculated to identify significant relationships ($r > 0.5$, $P < 0.05$) between floral shoot number and the relative expression level of each gene across treatments for a given week.

3. Results

3.1. Effects of temperature, soil moisture and light intensity on floral development

At the initiation of the experiment (week 0), all terminal buds were vegetative and producing new leaves; axillary buds were quiescent (inactive) (Fig. 2A). During the first 4 weeks of treatment, terminal buds of all trees continued vegetative shoot extension growth. Effects on shoot and leaf development due to different treatments were visible after 4 weeks. Shoot extension and leaf expansion were slower for LT-treated trees, resulting in smaller, less mature leaves at week 4 than trees in all other treatments (data not shown). Leaves of LLI-treated trees were larger than those of trees in other treatments. Symptoms of water-deficit stress appeared on developing shoots and young leaves of LSM-treated trees, including shoot tip browning and leaf necrosis.

At week 4, soil VWC was significantly lower for LSM-treated trees (< 10 %) compared to trees in all other treatments, which had soil VWC ≥ 20 % (Fig. 3A). During the subsequent 4 weeks, vegetative shoot extension growth ceased for trees in all treatments. By the end of the 8-week treatment period, terminal buds of LT-treated trees were at Stage 5 of inflorescence development, with separated bud scales evident (Fig. 2B); the four proximal axillary buds were at earlier stages of floral development (Fig. 2B). Although the internal anatomy of the buds was not analzyed in this study, according to the developmental scale of Salazar-García et al. (1998), terminal buds at Stage 5 are predictive of an inflorescence bud with a flat primary axis meristem and approximately 10 secondary axis inflorescence meristems. At the end of week 8 for LSM-, LLI- and OCG-treated trees, terminal buds were at Stage 1 of the developmental scale of Salazar-García et al. (1998), indicating the potential formation of two secondary axes inflorescence meristems based on this scale (Fig. 2C). Axillary buds remained quiescent (Fig. 2C). By week 12, four weeks after transfer of the trees from their respective environmental treatments to OGC, floral buds were visible on only the LT-treated trees. For these trees, the majority of terminal buds were at the early cauliflower stage of inflorescence development (Stage 8), and the four axillary buds were at Stages 5 and 6, according to the developmental scale of Salazar-García et al. (1998) (Fig. 2D). The terminal buds of LSM-, LLI- and OGC-treated trees remained at Stage 1, with quiescent axillary buds (Fig. 2C). During the subsequent two weeks of

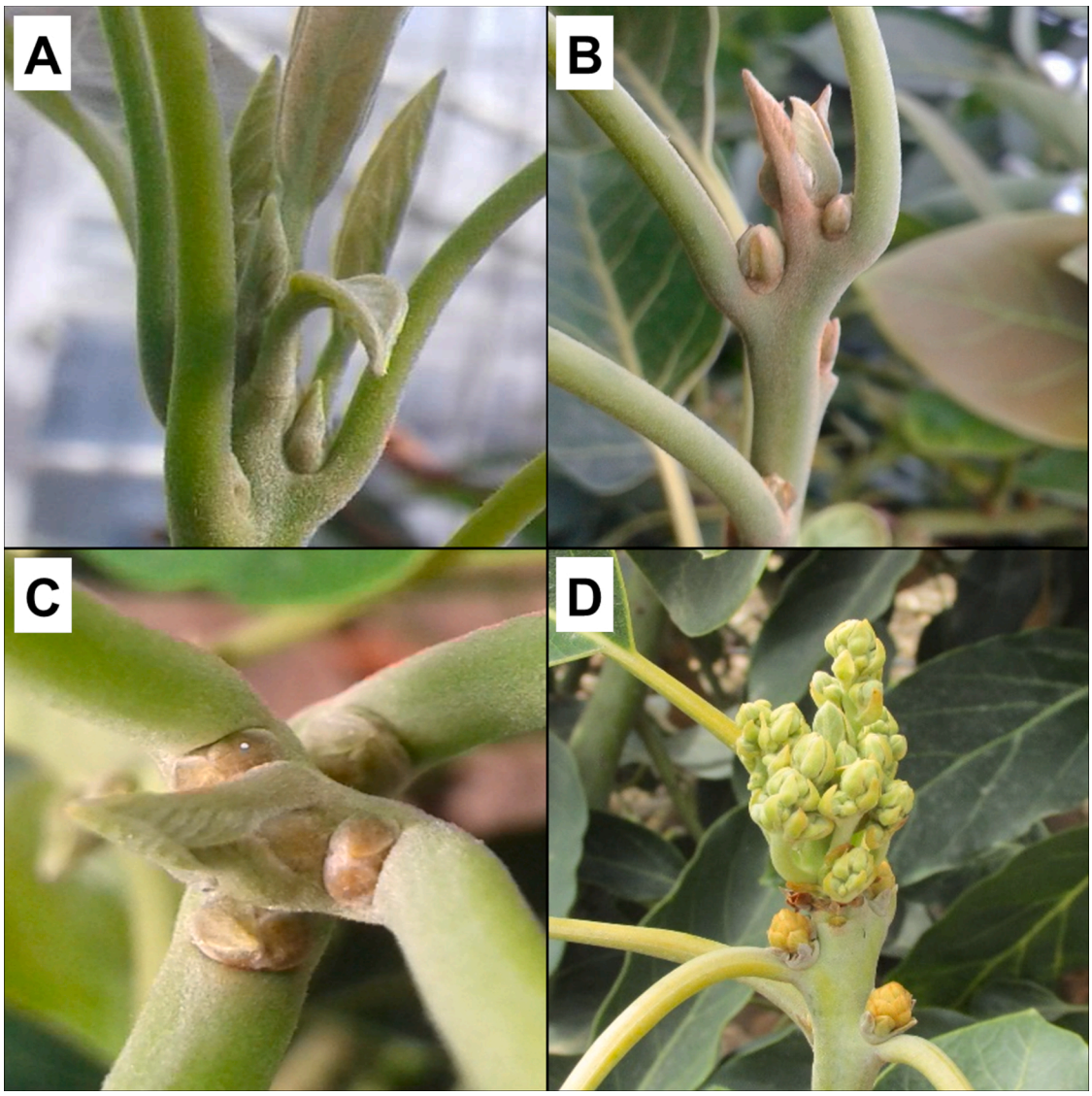

Fig. 2. Effect of environmental conditions on the developmental fate of 'Hass' avocado buds over time: (A) Week 0 - vegetative shoot bud; (B) Week 8 of LT treatment - terminal bud at Stage 5 of inflorescence development (axillary buds are at an earlier stage of inflorescence development); (C) Week 8 of LSM, LLI and OGC treatments - terminal bud at Stage 1 of inflorescence development; (D) Week 12 of LT treatment - terminal bud at the early cauliflower stage (Stage 8) and axillary buds at Stages 5 to 6 of inflorescence development. Reported stages of avocado bud floral development are based on the visual scale of Salazar-García et al. (1998).

OGC, floral development progressed only in buds of LT-treated trees; trees from the other environmental treatments never flowered. By week 14, 25.2 (84 %) of the 30 buds analyzed per LT-treated tree had produced floral shoots, all of which were indeterminate, with 0.03 (1 %) of the buds producing vegetative shoots, and 4.5 (15 %) remaining quiescent (inactive, did not produce a floral or vegetative shoot within the duration of the experiment) (Table 2). For LSM-, LLI- and OGC-treated trees, terminal buds remained at Stage 1; proximal axillary buds were quiescent.

Floral development in LT-treated trees was independent of water deficit. For LT-treated trees, soil VWC was approximately 20 % from weeks 2 through 8 of treatment, resulting in leaf midday water potentials $>$ –1.0 MPa during this period (Fig. 3A, B). In contrast, trees in the LSM treatment were subjected to 8 %–12 % soil VWC during weeks 2 through 8 and had significantly reduced leaf midday water potentials ($\leq$ –2.0 MPa) during this 6-week period (Fig. 3A, B). Although this is considered a moderate degree of water-deficit stress for promoting flowering in woody perennials (Lovatt et al., 1988; Chaikiattiyos et al., 1994), symptoms of water-deficit stress (as described above) were visible on young developing shoots and leaves of LSM-treated trees after only 4 weeks of treatment. Mature shoots were not damaged but browning and abscission of older leaves occurred. Thus, there remained many shoots with healthy viable buds. For trees in LLI and OGC treatments, soil VWC was $\geq$ 20 %, with leaf midday water potentials between –1.0 and –1.5 MPa for weeks 0 through 10. Thus, failure of trees in the LLI and OGC treatments to flower was not due to a negative effect of water deficit.

3.2. *Effects of temperature, soil moisture and light intensity on the bud expression of floral timing and floral meristem identity genes in 'Hass' avocado buds*

At week 0, the relative expression level of *Pa*FT was low (0.02) in buds of all trees (Fig. 4A). Bud expression of *PaFT* remained low for trees in all treatments with only two significant changes in expression, which occurred in weeks 10 and 12 of the experiment (2 and 4 weeks after the end of the environmental treatments and transfer of the trees to OGC, respectively) (Fig. 4A). For trees subjected to LSM, bud *PaFT* expression remained unchanged from week 0 through weeks 4 and 8, but decreased significantly in week 10 and remained low in week 12 (P = 0.008)

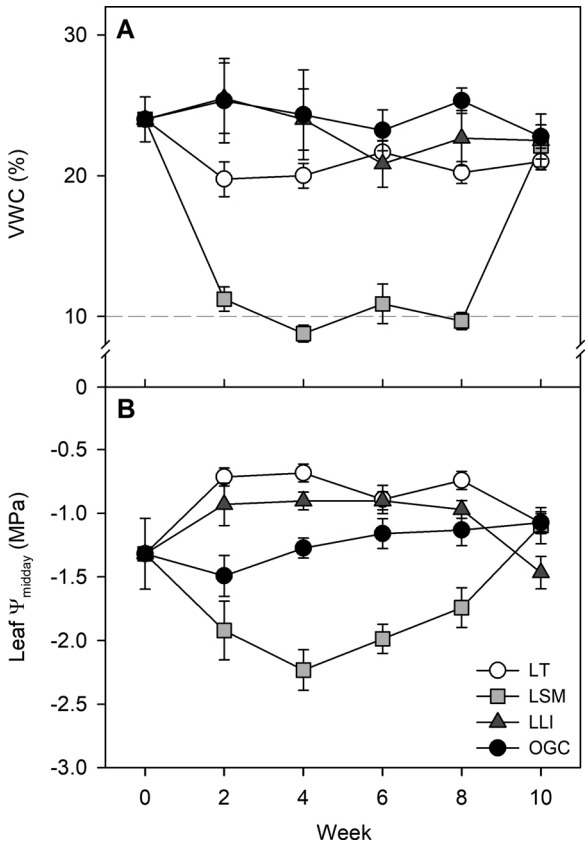

Fig. 3. (A) Soil moisture content reported as soil volumetric water content (% VWC) and (B) leaf midday water potential (MPa) for weeks 0 through 10 for trees subjected to 8 weeks of low temperature (LT), low soil moisture (LSM), or low light intensity (LLI) and then transferred to optimal growing conditions (OGC) for 6 weeks or maintained under OGC for 14 weeks. The horizontal dashed line in figure A is the target 10 % VWC set for the LSM treatment.

(Fig. 4A). In contrast, for LT-treated trees, bud transcript levels of *PaFT*, which were not significantly different in weeks 0, 4 and 8, increased from week 8 (end of LT treatment) to week 10 (2 weeks after transfer to OGC) ($P = 0.003$) to a value greater than that of trees in all other treatments ($P = 0.005$). Moreover, transcripts of *PaFT* continued to accumulate in buds of LT-treated trees after transfer to OGC, with the additional exposure to warm temperature, resulting in a 3-fold increase in *PaFT* transcript levels from week 10 to week 12 ($P < 0.003$). The resulting level of bud *PaFT* expression in week 12 was greater than that of trees in all other treatments ($P < 0.001$) (Fig. 4A).

In contrast, at week 0, *PaLFY* transcript levels were relatively high (3.0) in buds of trees in all treatments and continued to increase during the 8-week treatment period in buds of LT- and LSM-treated trees (5.8- and 4.2-fold, respectively) to levels greater than LLI- and OGC-treated trees by week 8 ($P = 0.002$) (Fig. 4B). Thus, *PaLFY* transcript levels increased in buds of LT-treated trees prior to any change in bud *PaFT* expression (Fig. 4A, B). By week 10, two weeks after transfer of all trees to OGC, *PaLFY* expression was significantly greater in buds of LT-treated trees than LSM- and OGC-treated trees, with *PaLFY* expression in buds of LLI-treated trees intermediate to, but not significantly different from, trees in all other treatments ($P = 0.009$) (Fig. 4B). By week 12 (4 weeks after transfer of all trees to OGC), bud expression of *PaLFY* in LT-treated trees decreased to values equal to those of LLI- and OGC-treated trees, but significantly greater than LSM-treated trees ($P = 0.006$) (Fig. 4B).

Bud transcript levels of *PaAP1* were not significantly different at week 0 and week 4 for trees in all treatments, except LT (Fig. 4C). Relative expression of *PaAP1* in buds of LT-treated trees increased 2-fold from week 0 to week 4 and 3-fold from week 0 to week 8 ($P = 0.085$) and was greater at week 4 ($P = 0.047$) and week 8 ($P < 0.001$) than that of trees in all other treatments (Fig. 4C). By week 10, bud *PaAP1* expression in LT-treated trees decreased to levels not significantly different from trees in other treatments, with the noted exception that buds of LSM-treated trees had *PaAP1* transcript levels significantly lower than buds of trees in all other treatments ($P = 0.030$) (Fig. 4C). Bud expression of *PaAP1* remained unchanged from week 10 to week 12 for LT-, LSM- and LLI-treated trees, but increased in OGC-treated trees ($P = 0.003$) to a value greater than that of trees in all other treatments ($P = 0.001$) (Fig. 4C).

For *PaAP2*, maximum transcript levels were detected in buds at week 0 (0.94) (Fig. 4D). For LLI- and OGC-treated trees, the variation in *PaAP2* expression over time was insignificant. However, bud expression of *PaAP2* was lower in LLI-treated trees at the end of the 8-week treatment compared to trees in all other treatments ($P = 0.001$) (Fig. 4D). After transfer of all trees to OGC, bud transcript levels of *AP2* were equal across treatments at week 10. By week 12, *PaAP2* expression was greater in buds of LLI- and OGC-treated trees than buds of LT- and LSM-treated trees ($P = 0.007$), due to the decrease in *PaAP2* transcript levels from week 8 to week 12 in buds of LT-treated ($P = 0.01$) and LSM-treated ($P < 0.001$) trees (Fig. 4D). Floral shoot number was strongly correlated across treatments with *PaAP1* at week 4 ($r = 0.99$, $P < 0.0001$) and week 8 ($r = 0.98$, $P < 0.05$) and *PaFT* at week 10 ($r = 0.98$, $P < 0.05$) and week 12 ($r = 0.99$, $P < 0.0001$).

3.3. *Effects of low temperature, low soil moisture and low light intensity on bud expression of floral organ identity genes in 'Hass' avocado*

The relative expression level of Pa*AP3* in buds remained low for trees in all treatments from week 0 through week 12, except for LT-treated trees (Fig. 5A). By week 12, *PaAP3* transcript accumulation in buds of LT-treated trees was greater than in previous weeks ($P = 0.003$) and all other treatments ($P < 0.001$) (Fig. 5A). For LSM- and LLI-treated trees, bud *PaAP3* expression decreased from week 10 to week 12 ($P = 0.002$). Buds of OGC-treated trees had an intermediate level of *PaAP3* expression in week 12 that was lower than that of LT-treated trees but greater than that of LSM- and LLI-treated trees ($P = 0.001$). Relative expression of *PaPI.1* in buds was low (0.01) at week 0, and remained low for trees under all environmental conditions over the 12-week experiment, except LT (Fig. 5B). Only buds of LT-treated trees accumulated *PaPI.1* transcripts over time ($P < 0.020$) to levels greater than those of trees in all other treatments by weeks 10 ($P = 0.002$) and 12 ($P < 0.001$) (Fig. 5B).

The expression pattern of *PaAG.1* in buds was similar to that of *PaPI.1*. Bud expression of *PaAG.1* was low (< 0.005) at week 0 and remained low in response to all environmental treatments over the 12-week experiment, with the exception of LT (Fig. 5C). Only buds of LT-treated trees accumulated *PaAG.1* transcripts over time ($P < 0.001$) to levels greater than those of trees in all other treatments in weeks 10 ($P = 0.002$) and 12 ($P < 0.001$). In contrast, bud expression of *PaAG.3* was not detected (ND) at week 0, and it fluctuated between not detected (ND) and low levels of expression across treatments over the 12-week experiment (Fig. 5D). At week 12, *PaAG.3* transcripts were below the limits of detection (ND) in the buds of LSM-, LLI- and OGC-treated trees, consistent with the fact that these trees did not produce flowers. In contrast, the LT treatment significantly increased bud *PaAG.3* transcript levels from week 10 to week 12 ($P = 0.037$). It is of significant interest that the expression of the floral organ identity genes increased only in buds of LT-treated trees and only after the LT-treated trees were transferred to OGC, which resulted in a significant increase in transcript levels from week 10 to week 12 for *PaAP3* ($P = 0.003$), *PaPI.1* ($P < 0.001$), *PaAG.1* ($P = 0.001$) and *PaAG.3* ($P = 0.037$); only the LT-treated trees flowered.

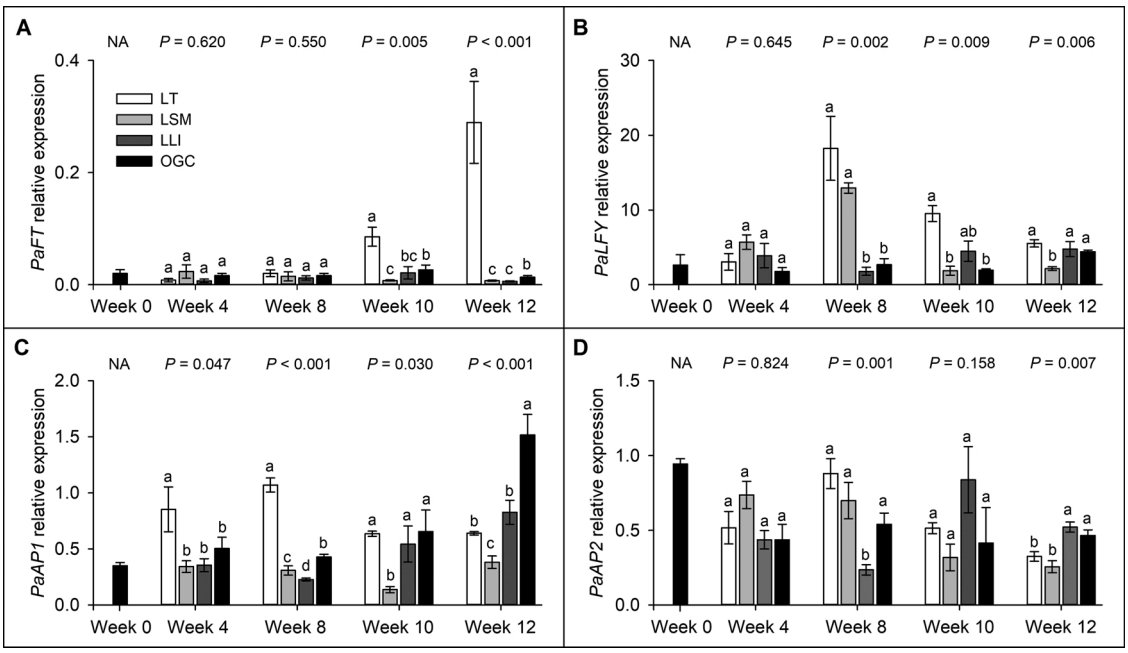

Fig. 4. Relative expression of (A) *PaFT*, (B) *PaLFY*, (C) *PaAP1* and (D) *PaAP2* in buds of 'Hass' avocado trees subjected to 8 weeks low temperature (LT) (10-hr day at 14 °C/14-hr night at 10°), low soil moisture (LSM) (soil VWC maintained between 8 % to 12 %), low light intensity (LLI) (PAR < 130 μmoles m^{-2} s^{-1}), or optimal growth conditions (OGC) (14-hr day at 30 °C, with PAR > 900 μmoles m^{-2} s^{-1}/10-hr night at 20 °C; soil VWC between 20 % to 25 %). At the end of 8 weeks of treatment, all trees were transferred to OGC for an additional 6 weeks. Data presented for week 0 represent the results of samples collected from trees at the start of the experiment (OGC, before trees were subjected to treatments), using four trees per sample to create three biological replications. ·All data are the means ± s.e. for three trees (replications) calculated relative to the expression of each target gene in 'Hass' avocado flowers (expression level = 1; normalized with *Paβ-ACTIN* expression) (Pfaffl, 2001); for the same week for a given gene, vertical columns with different lower-case letters are significantly different at the specified *P*-value according to Duncan's Multiple Range Test. NA refers to not applicable.

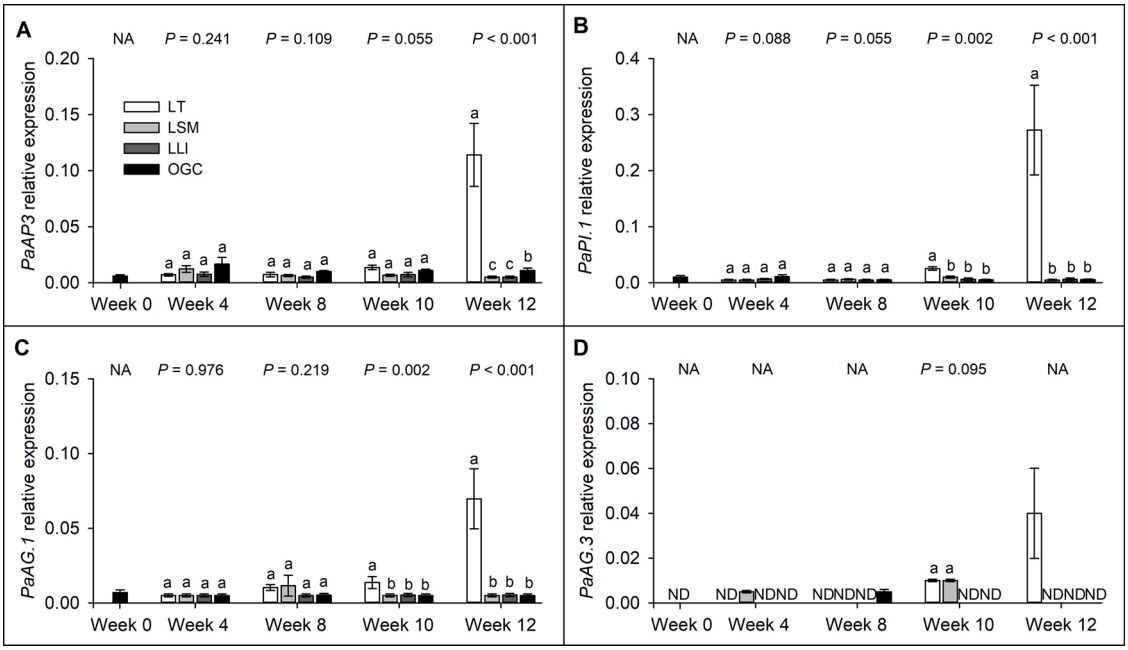

Fig. 5. Relative expression of (A) *PaAP3*, (B) *PaPI.1*, (C) *PaAG.1* and (D) *PaAG.3* in buds of 'Hass' avocado trees subjected to 8 weeks low temperature (LT) (10-hr day at 14 °C/14-hr night at 10°C), low soil moisture (LSM) (soil VWC maintained between 8 % to 12 %), low light intensity (LLI) (PAR < 130 μmoles m^{-2} s^{-1}), or optimal growth conditions (OGC) (14-hr day at 30 °C, with PAR > 900 μmoles m^{-2} s^{-1}/10-hr night at 20 °C; soil VWC between 20 % to 25 %). At the end of 8 weeks of treatment, all trees were transferred to OGC for an additional 6 weeks. Data presented for week 0 represent the results of samples collected from trees at the start of the experiment (OGC, before trees were subjected to treatments), using four trees per sample to create three biological replications.·All data are the means ± s.e. for three trees (replications) calculated relative to the expression of each target gene in 'Hass' avocado flowers (expression level = 1; normalized with *Paβ-ACTIN* expression) (Pfaffl, 2001); ND refers to not detected, which indicates the expression level of the target gene in each of the three biological replications was below the threshold value for detection (quantification cycle (Cq) in qPCR ≥ 35); for the same week for a given gene, vertical columns with different lower-case letters are significantly different at the specified *P*-value according to Duncan's Multiple Range Test; NS refers to not significant; NA refers to not applicable.

Table 2
Developmental fate of buds of 'Hass' avocado trees subjected to 8 weeks of low temperature (LT), low soil moisture (LSM), or low light intensity (LLI) and then transferred to optimal growth conditions (OGC) for 6 weeks or maintained under OGC for 14 weeks (treatment details are provided in Fig. 1).

Treatments	Floral shoots (no./tree)	Vegetative shoots (no./tree)	Quiescent buds (no./tree)
LT (Control)	25.2 a*	0.3 a	4.5 b
LSM	0.0 b	0.2 a	29.8 a
LLI	0.0 b	0.0 a	30.0 a
OGC	0.0 b	0.0 a	30.0 a
P-value	< 0.001	NS	< 0.001

* Values represent the mean for the distal five buds from six (nonbearing) shoots per tree (30 buds/tree) for each of six individual trees (replications) for each of the four treatments. Means within a vertical column followed by different lower-case letters are significantly different at the specified *P*-value using a pairwise *t*-test with Bonferroni adjustment.

4. Discussion

Flower development in 'Hass' avocado requires multiple steps. The first step is floral induction and transition of the vegetative SAM to an inflorescence meristem, which produces new lateral primordia that must acquire the identity of floral meristems (Benlloch et al., 2007). Genetic control of the induction process resulting in an inflorescence meristem, formation of floral meristems, initiation of floral organ primordia, floral organ specification and development of individual flowers is complex (Ma, 1994). Comparative studies suggest the genetic network regulating these processes is largely conserved among plant species (Benlloch et al., 2007). Thus in this research, core genes demonstrated to function in the network regulating floral development across many plant species were analyzed (Benlloch et al., 2007). In 'Hass' avocado, as in many woody perennials, the floral development process is protracted. Successful transition from a vegetative to reproductive SAM occurs many months prior to floral organogenesis and spring bloom. Environmental conditions at the time these events occur are not only critical to the reproductive success of a species but also to yield in a commercial tree crop, especially one like the 'Hass' avocado that relies on out-crossing (Hong and Jackson, 2015). Flowering must be timed to that of pollinizers and occur under environmental conditions that favor activity of pollinators, if required, and successful syngamy.

In this research, 'Hass' avocado trees subjected to 8 weeks of LT treatment flowered, a result consistent with earlier reports that various LT treatments, independent of photoperiod, promote flowering in 'Hass' and 'Fuerte' avocado (Buttrose and Alexander, 1978; Nevin and Lovatt, 1989; Chaikiattiyos et al., 1994; Salazar-García et al., 1999). The majority (84 %) of the buds analyzed for LT-treated trees produced indeterminate floral shoots. Comparison of the gene expression patterns in buds of trees subjected to four different environmental conditions provided strong evidence that accumulation of both *PaAP1* and *PaLFY* transcripts to maximum levels, which occurred in buds only after 8 weeks of LT treatment, were required and sufficient for irreversible commitment of the SAM to flowering in 'Hass' avocado. This interpretation is supported by the fact that transfer of LT-treated trees to the warm temperatures of the OGC did not prevent flowering but instead resulted in the activation of *PaFT* and subsequently the downstream floral organ identity genes with concomitant flowering. In contrast, buds of LLI- and OGC-treated trees failed to accumulate transcripts of either *PaLFY* or *PaAP1* to the level of LT-treated trees by the end of the 8-week treatment and did not flower. Even buds of LSM-treated trees, which accumulated transcript levels of *PaLFY* equal to those of LT-treated trees by week 8, but expressed lower levels of *PaAP1* than the buds of trees in all other treatments, did not flower. In *A. thaliana* and many other plant species, *LFY* and *AP1* are expressed in very early floral primordia, consistent with their role as key regulators of floral meristem identity (Blazquez et al., 2006; Sablowski, 2007, Benlloch et al., 2007; Hong and Jackson, 2015; Ma, 1994).

In light of the fact that the OGC-treated trees did not flower, bud expression of *PaLFY* (3.0), *PaAP1* (0.35) and *PaAP2* (maximum expression, 0.94) in these trees at the start of the experiment, following 5 months under OGC (week 0, July15), is of interest. In *A. thaliana*, bud expression of *LFY* and *AP1* is one of the first indications of the initiation of floral development (Melzer et al., 1999). The expression of *PaLFY* and *PaAP1* at the start of the experiment suggests the induction process might have been initiated, possibly by a signal (*PaFT*) from the leaves or an autonomous (endogenous developmentally regulated) floral development pathway that triggered the transition from vegetative to reproductive SAM, which occurs in summer in California (Salazar-García et al., 1998). However, since buds of LSM-, LLI- and OGC-treated trees subsequently expressed only low levels of *PaFT*, *PaAP3*, *PaPI.1* and *PaAG.1*, and did not express *PaAG.3* (ND) by week 12 and did not flower, the results suggest the process of induction might have been initiated but the resulting transcripts levels of *PaLFY* and *PaAP1* in the buds of these trees were insufficient at a critical stage of floral development to confer floral meristem identity and these trees did not flower. Consistent with this possibility, terminal buds of LSM-, LLI- and OGC-treated trees ceased vegetative shoot growth between weeks 4 and 8 and reached Stage 1 of inflorescence development according to the visual scale of Salazar-García et al. (1998) by week 8, but failed to develop further. In contrast, *PaLFY* and *PaAP1* expression continued to increase to maximum levels at week 8 of LT treatment that were sufficient to upregulate *PaFT*, *PaAP3*, *PaPI.1*, *PaAG.1* and *PaAG.3*, and result in flowering.

Ziv et al. (2014) provided the first evidence suggesting that leaf expression of *PaFT* initiated the floral induction process in 'Hass' avocado under low temperature conditions, resulting in the upregulation of *PaLFY* and *PaAP1* expression in buds, analogous to initiation of floral development in *A. thaliana.* The continued increase in *PaAP1* transcript levels after 4 and 8 weeks of LT treatment followed by increased expression of *PaLFY* after 8 weeks of LT is consistent with these results. In addition, the results of the present research provided strong evidence that bud expression of both *PaAP1* and *PaLFY* at sufficient levels is required to achieve floral meristem identity. The LSM treatment resulted in activation of bud *PaLFY* but not *PaAP1*, and the trees did not flower. Additionally, Ziv et al. (2014) first reported the late expression of *PaFT* in 'Hass' avocado buds after the upregulation of *PaLFY* and *PaAP1* expression and after formation of two secondary axis inflorescence meristem in buds, and proposed the possible involvement of *PaFT* in flower developmental processes in the avocado bud prior to anthesis. Results presented herein also demonstrated the late expression of *PaFT* in weeks 10 and 12 after the upregulation of *PaAP1* and *PaLFY* in 'Hass' avocado buds that went onto flower, i.e., buds of LT-treated trees. Thus, transcript accumulation of *PaFT* occurred 2 and 4 weeks after transfer of the LT-treated trees to OGC in contrast to the results of Ziv et al. (2014), where increased bud expression of *PaFT* occurred under low temperature conditions of late fall. The results of the research presented herein documented that the late upregulation of *PaFT* in avocado buds 2 and 4 weeks after transfer of LT-treated trees to OGC (weeks 10 and 12) was followed by activation *PaAP3*, *PaPI.1*, *PaAG.1* and *PaAG.3* in week 12, and subsequent flowering, providing new evidence in support of the second role of *PaFT* in successful flowering in the 'Hass' avocado and new insight into *PaFT* function. In *A. thaliana, FT* has a second role, independent of its primary function in floral induction, to maintain floral meristem determinacy and prevent floral reversion, which is essential for the full development of the inflorescence, especially one that develops acropetally to ensure the development of a terminal flower (Parcy et al., 2002; Müller-Xing et al., 2014). Moreover, in *A. thaliana*, *AP1* and *LFY* also have primary and secondary roles. The first is in bud determinacy and the second in floral organ specification; *AP1* in the development of the perianth organs (sepals and petals) and *LFY* in the activation of genes specifying stamen and carpel development

(Sablowski, 2007). Similarly, for avocado, results suggest that *PaAP1*'s primary role is in bud determinacy, but *PaAP1* is also expressed in the perianth organs (i.e., in both whorls of tepals) and in the stamens (Chanderbali et al., 2006). A second role for *PaLFY* in floral organogenesis has not been reported in *P. americana*, but bud *PaLFY* expression remained elevated concurrently with increased bud *PaFT* expression in week 10 of LT-treated trees.

The results reported herein, to the authors' knowledge, are the first data on the expression of *PaAP2* in buds of *P. americana*. Surprisingly, maximum bud expression of *PaAP2* was in week 0. The expression patterns of *AP2* did not vary significantly over time or in response to different environmental conditions. In *A. thaliana*, *AP2* has class A function and a role in sepal development (Bowman et al., 1991; Coen and Meyerowitz, 1991; Krizek and Fletcher, 2005). The results of this research do not clarify the role of *PaAP2* in avocado floral development.

Maximum expression of floral organ identity genes *PaAP3*, *PaPI.1*, *PaAG.1* and *PaAG.3* in 'Hass' avocado buds occurred 4 weeks after transfer of LT-treated trees to OGC (week 12). At this time, terminal buds were at Stage 8, cauliflower stage of inflorescence development, in which all floral organs are present and during which the final stages of pollen and ovule development occur (Salazar-García et al., 1998). The four proximal axillary buds were at stages 5 and 6, with perianth development initiated and the perianth fully formed, respectively (Salazar-García et al., 1998). In *P. americana*, *PaAP3*, *PaPI.1* and *PaAG.1* are expressed in the outer and inner tepals and stamens; *PaAG.3* is expressed in the stamens and carpel (Chanderbali et al., 2006). For LSM-, LLI- and OGC-treated trees at week 12, bud transcript levels of *PaAP3*, *PaPI.1*, *PaAG.1* and *PaAG.3*, in all cases, were unchanged or lower than in week 0, remaining at low levels or below the limits of detection (ND) (*AG.3*). LSM-, LLI- and OGC-treated trees did not flower. Terminal buds were at Stage 1 and proximal axillary buds remained quiescent (Salazar-García et al. (1998). In field-grown 'Hass' avocado trees in California and Florida, terminal buds reach Stage 8 in March (Salazar-García et al., 1998; Buzgo et al., 2007).

The results of this research provide new information regarding the effects of two additional environmental factors on 'Hass' avocado flowering. Herein we documented that 6 weeks of low soil moisture (8 %–12 % VWC), resulting in leaf midday water potentials $\leq$ -2.0 MPa and visible symptoms of moderate water-deficit stress sufficient to promote flowering in citrus (Southwick and Davenport, 1986; Lovatt et al., 1988), failed to induce flowering in avocado. Whereas the LSM treatment upregulated *PaLFY* expression to levels equal to buds of LT-treated trees, it failed to activate *PaAP1*. The results provided new evidence that low light intensity (PAR $<$ 130 μmoles m^{-2} s^{-1}) failed to increase both *PaLFY* and *PaAP1* expression sufficiently to result in floral determinacy in avocado. Based on these results, during the period of 'Hass' avocado floral induction, low soil moisture causing moderate water-deficit stress to the tree, and low light intensity in the canopy, that can result from overgrowth within and across orchard rows, should be prevented. The results also ruled out the possibility that plant water deficit was the factor limiting flowering in LLI- or OGC-treated trees or promoting flowering in LT-treated trees. Leaf midday water potentials for these trees were $>$ -1.5 MPa for weeks 0 through 10. It should be noted that OGC-treated trees were maintained under these conditions continuously for 8.5 months and did not flower.

The induction of flowering in 'Hass' avocado by LT, but not water deficit, was also documented for longan (*Dimocarpus longan*) (Chen et al., 2010). However, it is in contrast to *Citrus* spp., in which flowering is promoted by both LT and water deficit, singularly and in combination. For *Citrus* spp., the lack of sufficient LT during the winter in tropical and semi-tropical growing-areas is supplemented by water deficit to ensure adequate flowering for commercial-level crop production (Southwick and Davenport, 1986; Lovatt et al., 1988; Nakajima et al., 1992). The results of this research indicate that subjecting 'Hass' avocado trees to water deficit is not an option for increasing flowering in warm winters. Also, it will be possible to use water deficit to promote out-of-season avocado flowering and fruit production as is done with lemons (*C. limon*) and limes (*C. latifolia*) (Lovatt et al., 1988). The results of this research do not clarify whether water deficit simply fails to induce flowering or actually inhibits a critical step in the floral development process. Thus, the impact of imposing varying degrees of water deficit during LT-floral inductive conditions on avocado flowering is not known. In the absence of such information, 'Hass' avocado growers would be wise to err on the side of caution and prevent water deficit during floral induction and completion of the floral development process.

The results presented herein provided new evidence demonstrating that upregulation of both *PaLFY* and *PaAP1* in 'Hass' avocado buds was required for floral meristem determinacy. Consistent with successful induction by LT treatment, flowering was not prevented by the transfer of the 8-week LT-treated trees to OGC prior to *PaFT* activation, which did not occur by week 8 but was evident in week 10 (2 weeks after transfer to OGC), suggesting that the expression of *PaFT* in avocado buds might not be involved with floral induction. Instead, the results provided evidence consistent with a role for *PaFT* in maintenance of avocado floral meristem determinacy during the process of acropetal inflorescence development and flower formation in 'Hass' avocado.

In 'Hass' avocado, four weeks after the LT-treated trees were transferred to OGC, bud expression levels of *PaFT* and the floral organ identity genes *PaAP3*, *PaPI.1*, *PaAG.1* and *PaAG.3* reached their maximum. The fact that bud expression of *PaFT*, *PaAP3*, *PaPI.1*, *PaAG.1* and *PaAG.3* did not occur until after transfer of the LT-treated trees to OGC suggests a possible failsafe mechanism in avocado to synchronize flowering induced by lower fall and winter temperatures with the warmer temperatures of spring. Transcripts of *PaLFY* and *PaAP1* would accumulate in buds under low temperatures during fall and winter to a level sufficient to confer floral meristem identity, but bud *PaFT*, which our results suggest plays a role in maintaining commitment to flowering in 'Hass' avocado, and the downstream floral organ identity genes *PaAP3*, *PaPI.1*, *PaAG.1* and *PaAG.3* would only be fully expressed in 'Hass' avocado buds when spring temperatures were sufficiently warm, thereby preventing formation of the flowers necessary to complete inflorescence development from occurring under adverse temperature conditions.

Funding information

This research was supported in part by the Citrus Research Center and Agricultural Experiment Station of the University of California, Riverside, California. A. Acosta-Rangel was supported in part by Minciencias – Colombia (Grant number529). This paper represents a portion of the dissertation submitted by A. Acosta-Rangel in partial fulfilment of the requirements for a Ph.D. in Plant Biology at the University of California.

CRediT authorship contribution statement

Aleyda Acosta-Rangel: Conceptualization, Investigation, Writing - original draft, Methodology, Data curation, Formal analysis, Methodology. **Rui Li:** Methodology. **Peggy Mauk:** Supervision, Funding acquisition. **Louis Santiago:** Supervision, Funding acquisition. **Carol J. Lovatt:** Visualization, Conceptualization, Investigation, Data curation, Writing - original draft, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors report no declarations of interest.

Acknowledgements

The authors thank Drs. Samuel Salazar-García for reviewing the bud development data and the manuscript, Ben Faber for reviewing the

manuscript, and Amy Litt and Marta Ruiz for their useful suggestions during the research.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.scienta.2021.109940.

References

Benlloch, R., Berbel, A., Serrano-Mislata, A., Madueno, F., 2007. Floral initiation and inflorescence architecture: a comparative view. Ann. Bot. 100, 659–676.

Blanke, M.M., Lovatt, C.J., 1992. Anatomy and transpiration of the avocado inflorescence. Ann. Bot. 71, 543–547.

Blazquez, M.A., Ferrandiz, C., Madueno, F., Parcy, F., 2006. How floral meristems are built. Plant Mol. Biol. 60, 855–870.

Bowman, J.L., Smyth, D.R., Meyerowitz, E.M., 1991. Genetic interactions among floral homeotic shoots by removal of terminal buds early in the flower bud induction period. Proc. Florida State Hort. Soc. 124, 60–64.

Buttrose, M.S., Alexander, D.M.E., 1978. Promotion of floral initiation in 'Fuerte' avocado by low temperature and short daylength. Sci. Hort. 8, 213–217. https://doi.org/10.1016/0304-4238(78)90027-4.

Buzgo, M., Chanderbali, A.S., Kim, S., 2007. Floral development morphology of *Persea americana* (avocado, Lauraceae): the oddities of male organ identity. Intl. J. Plant Sci. 168, 261–284.

Chaikiattiyos, S., Menzel, C.M., Rasmussen, T.S., 1994. Floral induction in tropical fruit trees: effects of temperature and water supply. J. Hort. Sci. 69, 397–415.

Chanderbali, A.S., Kim, S., Buzgo, M., 2006. Genetic footprints of stamen ancestors guide perianth evolution in *Persea* (Lauraceae). Intl. J. Plant Sci. 167, 1075–1089. https://doi.org/10.1086/507586.

Chanderbali, A.S., Albert, V.A., Ashword, V.E.T.M., 2008. *Persea americana* (avocado): bringing ancient flowers to fruit in the genomics era. BioEssays 30, 386–396.

Chanderbali, A.S., Albert, V.A., Leebens-Mack, J., Altman, N.S., Soltis, D.E., Soltis, P.S., 2009. Transcriptional signatures of ancient floral developmental genetics in avocado (*Persea americana*; Lauraceae). Proc. Natl. Acad. Sci. U. S. A. 106, 8929–8934.

Chanderbali, A.S., Yoo, M.-Y., Zahn, L.M., 2010. Conservation and canalization of gene expression during angiosperm diversification accompany the origin and evolution of the flower. Proc. Natl. Acad. Sci. U. S. A. 107, 22570–22575.

Chanderbali, A.S., Berger, B.A., Howarth, D.G., Soltis, P.S., Soltis, D.E., 2016. Evolving ideas on the origin and evolution of flowers: new perspectives in the genomic era. Genetics 202, 1255–1265.

Chen, Q.X., Pan, D.M., Lu, L.X., 2010. Effect of temperature on flower-bud induction of longan in phytotron. Acta. Hort. 863, 279–284.

Coen, E.S., Meyerowitz, E.M., 1991. The war of the whorls: genetic interactions controlling flower development. Nature 353, 31–37.

Hoagland, D.R., Arnon, D.I., 1950. The water culture for growing plants without soil. Calif. Agri. Exp. Stat. Circular 347 revised.

Hong, Y., Jackson, S., 2015. Floral induction and flower formation–the role and potential applications of miRNAs. Plant Biotechnol. J. 13, 282–292.

Integrated DNA Technologies (IDT), 2018. PrimerQuest Tool. Accessed July 6, 2018. https://www.idtdna.com/pages.

Krizek, B.A., Fletcher, J.C., 2005. Molecular mechanisms of flower development: an armchair guide. Nat. Rev. Gen. 6, 688–698.

Lee, J., Lee, I., 2010. Regulation and function of *SOC1*, a flowering pathway integrator. J. Exp. Bot. 61 (9), 2247–2254, 2010 doi:10.1093/jxb/erq098.

Lovatt, C.J., Zheng, Y., Hake, K.D., 1988. Demonstration of a change in nitrogen metabolism influencing flower initiation in *Citrus*. Israel J. Bot. 37 (2–4), 181–188. https://doi.org/10.1080/0021213X.1988.10677098.

Ma, H., 1994. The unfolding drama of flower development: recent results from genetic and molecular analyses. Genes Dev. 8, 745–756.

Matasci, N., Hung, L.-H., Yan, Z., 2014. Data access for the 1,000 Plants (1KP) project. Gigascience 3, 17. https://doi.org/10.1186/2047-217X-3-17.

Melzer, S., Kampmann, G., Chandler, J., Apel, K., 1999. *FPF1* modulates the competence to flowering in *Arabidopsis*. Plant J. 18, 395–405.

Moon, J., Lee, H., Kim, M., Lee, I., 2005. Analysis of flowering pathway integrators in *Arabidopsis*. Plant Cell Physiol. 46, 292–299. https://doi.org/10.1093/pcp/pci024.

Müller-Xing, R., Clarenz, O., Pokorny, L., Goodrich, J., Schubert, D., 2014. Polycomb-group proteins and *FLOWERING LOCUS T* maintain commitment to flowering in *Arabidopsis thaliana*. Plant Cell 26, 2457–2471.

Nakajima, Y., Susanto, S., Hasegawa, K., Agriculture, F., 1992. Influence of water stress in autumn on flower induction and fruiting in young pomelo trees (*Citrus grandis* L Osbeck). J. Japan. Soc. Hort. Sci. 62 (1), 15–20.

Nevin, J.M., Lovatt, C.J., 1989. Changes in starch and ammonia metabolism during low temperature stress-induced flowering in 'Hass' avocado - a preliminary report. South Afr. Avocado Growers' Assoc. Yrbk. 12, 21–25.

Núñez-Elisea, R., Davenport, T.L., 1994. Flowering of mango trees in containers as influenced by seasonal temperature and water stress. Sci. Hort. 58, 57–66. https://doi.org/10.1016/0304-4238(94)90127-9.

Parcy, F., 2005. Flowering: a time for integration. Int. J. Dev. Biol. 49, 585–593. https://doi.org/10.1387/ijdb.041930fp.

Parcy, F., Bomblies, K., Weigel, D., 2002. Interaction of *LEAFY*, *AGAMOUS* and *TERMINAL FLOWER1* in maintaining floral meristem identity in *Arabidopsis*. Development 129, 2519–2527.

Pfaffl, M.W., 2001. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res. 29, 2002–2007.

R Core Team, 2013. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. URL. http://www.R-project.org/.

Ratcliffe, O., Bradley, D., Coen, E., 1999. Separation of shoot and floral identity in *Arabidopsis*. Development 126, 1109–1120.

Sablowski, R., 2007. Flowering and determinacy in *Arabidopsis*. J. Exp. Bot. 58, 899–907.

Salakpetch, S., Turner, D.W., Dell, B., 1990. The flowering of carambola (*Averrhoa carambola* L.) is more strongly influenced by cultivar and water stress than by diurnal temperature variation and photoperiod. Sci. Hort. 43, 83–94.

Salazar-García, S., Lord, E.M., Lovatt, C.J., 1998. Inflorescence and flower development of the 'Hass' Avocado (*Persea americana* Mill.) during "on" and "off" crop years. J. Am. Soc. of Hort. Sci. 123, 537–544.

Salazar-García, S., Lord, E.M., Lovatt, C.J., 1999. Inflorescence development of the 'Hass' avocado: commitment to flowering. J. Am. Soc. Hortic. Sci. 124, 478–482.

Shen, J., Xiao, Q., Qiu, H., Chen, C., Chen, H., 2016. Integrative effect of drought and low temperature on litchi (*Litchi chinensis* Sonn.) floral initiation revealed by dynamic genome-wide transcriptome analysis. Sci. Rep. 6, 32005 doi: 1038/srep32005.

Siriwardana, N., Lamb, R., 2012. The poetry of reproduction: the role of *LEAFY* in *Arabidopsis thaliana* flower formation. Int. J. Dev. Biol. 56, 207–221. https://doi.org/10.1387/ijdb.113450ns.

Soltis, D.E., Chanderbali, A.S., Kim, S., Buzgo, M., Soltis, P.S., 2007a. The ABC model and its applicability to basal angiosperms. Ann. Bot. 100, 155–163. https://doi.org/10.1093/aob/mcm117.

Soltis, D.E., Ma, H., Frohlich, M.W., 2007b. The floral genome: an evolutionary history of gene duplication and shifting patterns of gene expression. Trends Plant Sci. 12, 358–367.

Southwick, S.M., Davenport, T.L., 1986. Characterization of water stress and low temperature effects on flower induction in *Citrus*. Plant Physiol. 81, 26–29. https://doi.org/10.1104/pp.81.1.26.

Stern, R.A., Adato, I., Goren, M., Eisenstein, D., Gazit, S., 1993. Effects of autumnal water stress on litchi flowering and yield in Israel. Sci. Hort. (Amsterdam) 54, 295–302. https://doi.org/10.1016/0304-4238(93)90108-3.

Takeno, K., 2016. Stress-induced flowering: the third category of flowering response. J. Exp. Bot. 67 (17), 4925–4934. https://doi.org/10.1093/jxb/erw272.

Ziv, D., Zviran, T., Zezak, O., Samach, A., Irihimovitch, V., 2014. Expression profiling of *FLOWERING LOCUS T*-like gene in alternate bearing 'Hass' avocado trees suggests a role for *PaFT* in avocado flower induction. PLoS One 9. https://doi.org/10.1371/journal.pone.0110613.

National Center for Biotechnology Information (NCBI). Bethesda (MD): National Library of Medicine (US).