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Application of an Analog of 9, 10-ketol-octadecadienoic acid (KODA), Affected Flower Bud Formation and *MdTFL1* and *MdFT1* Gene Expressions in Apple Buds under Heavy-crop and Shade Conditions

Monrudee Kittikorn, Katsuya Okawa, Hitoshi Ohara, and Satoru Kondo[1]
Graduate School of Horticulture, Chiba University, Matsudo 271-8510, Japan

Nobuhiro Kotoda and Masato Wada
National Institute of Fruit Tree Science, Morioka 020-0123, Japan

Mineyuki Yokoyama and Ohji Ifuku
Technology and Engineering Center, Shiseido Co., Yokohama 236-8643, Japan

Ariake Murata and Naoharu Watanabe
Graduate School of Science and Technology, Shizuoka University, Shizuoka 422-8529, Japan

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Abstract. **Changes of endogenous 9, 10-ketol-octadecadienoic acid (KODA) concentrations, which is synthesized from linolenic acid by 9-lipoxygenase, were analyzed in apple [*Malus* ×*sylvestris* (L.) Mill. var. *domestica* (Borkh.) Mansf.] buds. In addition, the effects of 15, 16-chloro, hydroxy-9-hydroxy-10-oxo-12(Z)-octadecenoic acid (CKODA) application, which is an analog of KODA, on flower bud formation and the expression of *MdTFL1* (*terminal flower 1*) and *MdFT1* (*flowering locus t 1*) genes in apple buds were investigated in heavy-crop treatment (HCT) and under shade. An increase of endogenous KODA in the buds in the fruit-thinning treatment, which resulted in a higher proportion of flower bud formation than in HCT, was observed at 63 days after full bloom, but no such increase was found in HCT. In the shade-treated and heavy-crop trees, the expression of *MdTFL1* in the buds to which CKODA was applied was lower than that in untreated buds. In contrast, under shade, the expression of *MdFT1* in the CKODA-treated buds was higher than that in untreated buds. These results suggest that endogenous KODA may be associated with flower bud formation, and its application may be effective at improving the proportion of flower bud formation through its effect on *MdTFL1* and *MdFT1*.**

Most fruit trees such as apple trees have a long vegetative period, which is undesirable for growers (Foster et al., 2003; Mimida et al., 2009). In the adult phase, alternate bearing is induced by growing conditions such as heavy fruit load that is associated with gibberellins (Ramirez et al., 2001, 2004). Therefore, the clarification of the physiological mechanism for regulation of flower bud formation is indispensable for stable apple production.

Environmental conditions such as light intensity also influence floral initiation in apple. For instance, low irradiance has been shown to inhibit floral initiation in apical buds of 'Fuji' apple (Kittikorn et al., 2011). The flower inductions of duckweed (*Lemna paucicostata* L.) and japanese morning glory (*Pharbitis nil* Choisy) were shown to be associated with KODA levels (Suzuki et al., 2003; Yokoyama et al., 2000). Furthermore, it was shown that endogenous KODA concentrations in apical buds were higher in trees with a higher flower bud formation, and that KODA spraying of one-year-old shoots increased the proportion of flower bud formation in trees with normal fruit load [40 leaves per fruit (Kittikorn et al., 2010, 2011)]. These facts show that KODA may be associated with flower bud formation in apple.

Studies on the molecular mechanisms of flowering have been well established in arabidopsis [*Arabidopsis thaliana* (L.) Heynh. (Bradley et al., 1997)]. Numerous floral meristem identity genes have been isolated from arabidopsis, and several models for the genetic pathways of floral induction and differentiation such as *FT*, *suppressor of constans 1* (*SOC1*), *leafy* (*LFY*), *apetala 1* (*AP1*), and *TFL1* have been reported (Henderson and Dean, 2004; Kobayashi et al., 1999; Ratcliffe et al., 1998). Overexpression of *LFY*, *FT*, or *AP1* has been shown to shorten the vegetative phase, causing early flowering (Kardailsky et al., 1999), whereas overexpression of *TFL1* has been shown to cause late flowering in transgenic arabidopsis (Ratcliffe et al., 1998). *TFL1* is a key gene involved in repression flowering and maintains the identity of inflorescence meristems by preventing the expression of *AP1* and *LFY* (Ratcliffe et al., 1998). Arabidopsis plants in which the *MdTFL1* gene was transferred showed delayed flowering (Kotoda and Wada, 2005), but 'Orin' apple plants with transferred antisense *MdTFL1* RNA flowered after eight months compared with the untreated control

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[1] Corresponding author. E-mail: s-kondo@faculty.chiba-u.jp.

plants, which took six years to flower. In addition, *MdTFL1* was detected in vegetative tissues such as apical buds, stems, and cotyledon, but not in reproductive tissues such as floral organs (sepal, petal, and stamen), suggesting its key role in maintaining the juvenile and/or vegetative phase in apple (Kotoda et al., 2006). Contrary to the functional role of *TFL1*, *FT* promoted flowering by altering the expression of other endogenous genes (Esumi et al., 2007; Kardailsky et al., 1999). Invariably, overexpression of *FT*-like genes has led to premature flowering under non-inductive conditions in species such as arabidopsis (Abe et al., 2005), rice [*Oriza sativa* L. (Tamaki et al., 2007)], tomato [*Solanum lycopersicum* L. (Lifschitz et al., 2006)], and grape [*Vitis vinifera* (Carmona et al., 2007)]. KODA application decreased *MdTFL1* expression in treated buds (Kittikorn et al., 2011). Therefore, it is possible that KODA application induces flower bud formation in apple through an effect on *MdTFL1* transcription. An investigation of the relationship among KODA, *MdTFL1,* and *MdFT1* under various growing conditions may clarify the roles of KODA. However, the effect of KODA application under non-inductive conditions has not been examined.

We have found that CKODA (Fig. 1), an analog of KODA, was more stable than KODA at high temperatures (Kondo et al., 2012). This study examined the effect of CKODA application on the transcription of *MdTFL1* and *MdFT1* and flower bud formation in apple under heavy cropload and shade conditions.

Materials and Methods

Plant materials. Experiments were conducted in 2009 and 2010 on 'Fuji' apple trees grafted onto 'Marubakaido' rootstock (*Malus prunifolia* Borkh. var. *ringo* Asami) growing in an open field of Chiba University located at lat. 36° N and long. 139° E at an altitude of 747 m.

Expt. 1: effect of crop load on the changes of endogenous KODA. Two groups of three 32-year-old trees (three replications of one tree per each treatment) were created randomly in 2009: one that underwent flower thinning treatment (FTT), in which all flowers were removed at full bloom, and one that underwent HCT, in which the number of leaves per fruit was adjusted to 20 at 20 d after full bloom (DAFB). At 21, 49, 63, and 103 DAFB, 90 terminal buds from shoot meristems (three replications of 30 buds per tree) were randomly collected for analysis of endogenous KODA from each treatment. The sampled buds were from non-fruiting spurs in each treatment.

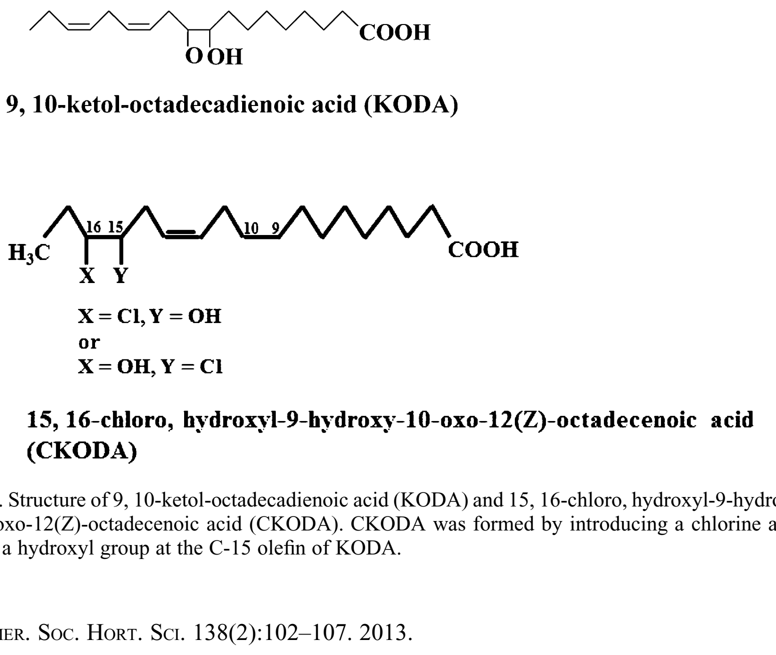

Fig. 1. Structure of 9, 10-ketol-octadecadienoic acid (KODA) and 15, 16-chloro, hydroxyl-9-hydroxy-10-oxo-12(Z)-octadecenoic acid (CKODA). CKODA was formed by introducing a chlorine atom and a hydroxyl group at the C-15 olefin of KODA.

Analysis of endogenous KODA. The buds, including whole apical tissues (100 mg fresh weight per 30 buds in one tree from each treatment), were ground in liquid nitrogen and extracted with methanol (10 mL) using [U-^{13}C] KODA (1 pmol·μL^{-1}) as an internal standard at 4 °C. The filtrate was concentrated in vacuo and the concentrates were re-dissolved in methanol (5 mL) and washed with hexane (10 mL) to remove pigments. The methanol layer thus obtained was evaporated in vacuo to yield solid materials. The resultant solid materials were re-dissolved in 100 μL ethanol, and 5 μL of the solution was subjected to high-performance liquid chromatography-mass spectrometry (HPLC-MS/MS) (LTQ-Orbitrap Discovery; Thermo Fisher Scientific, Bremen, Germany) equipped with a Capcell Pak C18 UG 120 column (2 mm i.d. × 7.5 cm; Shiseido, Tokyo, Japan) that was maintained at 4 °C . The samples were analyzed at a flow rate of 0.2 mL·min^{-1} using a chromatographic gradient of two mobile phases (A = 0.05% aqueous formic acid, B = acetonitrile). Solvent B was linearly increased from 15% to 100% over 15 min.

The MS conditions were set as follows: ionization mode, electron spray ionization (negative); capillary temperature, 330 °C; capillary voltage, –27.50 V; collision energy, 35.0 eV; and full mass range, *m/z* 50 to *m/z* 350. MS^2 values were acquired from a parent ion at *m/z* 309 [M-H]$^-$ for KODA with a scan range of *m/z* 85 to *m/z* 310. The MS^2 spectra of [U-^{13}C] KODA were determined based on a selection reaction monitoring an ion at *m/z* 327 [M-H]$^-$ with a scan range of *m/z* 263.5 to *m/z* 264.5. [U-^{13}C]KODA was eluted at t_R = 10.47 min with an ion peak at *m/z* 327 [M-H]$^-$. The MS^2 spectrum from the ion at *m/z* 327 exhibited ion peaks at *m/z* 309 (100%) [M-H$_2$O]$^-$ and *m/z* 264 (68.0%) [M-H$_2$O-H^{13}COO]$^-$. The recovery of KODA was estimated based on the ion intensity at *m/z* 309. Similarly, KODA was detected at t_R = 10.40 min with an ion peak at *m/z* 309. The MS^2 spectrum from the ion at *m/z* 309 showed ion peaks at *m/z* 291 (100%) [M-H$_2$O] and *m/z* 247 (72.3%) [M-H$_2$O-HCOO]$^-$. Although 13-α-ketol had the same retention time and ion peak at *m/z* 309 [M-H]$^-$ as those of KODA, the MS^2 spectrum of 13-α-ketol was recorded with ion peaks at *m/z* 291 (100%) [M-H$_2$O]$^-$ and *m/z* 247 (3.1%) [M-H$_2$O-HCOO]$^-$. Furthermore, the ionization efficacy for *m/z* 291 of 13-α-ketol was 3.56 times higher than that of KODA. Quantification of KODA was based on the ion intensities of *m/z* 247 and *m/z* 291 of KODA and 13-α-ketol.

Expt. 2: Effect of CKODA application on the expression of *MdTFL1* and *MdFT1* genes under heavy-crop and shade conditions. In the 2010 HCT, four 33-year-old trees were randomly selected. The number of leaves per fruit was adjusted to 20 at 20 DAFB as in Expt. 1. One hundred forty one-year-old shoots (560 apical buds in four trees), also randomly selected at 7 DAFB, in each tree were sprayed with 100 μM CKODA with surfactant 0.1% (v/v) (New Osmac®; Hayashi Chemical, Tokyo, Japan) at 7, 21, 35, and 49 DAFB (HCT$^+$CKODA$^+$). On each day of

Table 1. Gene-specific primer sets used for quantitative real-time reverse transcription–polymerase chain reaction analyses of *MdTFL1* and *MdFT1* in apical buds of 'Fuji' apple.

Gene	Forward primer (5′–3′)	Reverse primer (5′–3′)
MdTFL1	TGTTGTCACAGCCAAACCTAG	ACAATCCAGTGCAGGTGCTCC
MdFT1	CTTGTTGTTGGACGAGTGG	GAGTGTAGAAAGTCCTGAGAT
Actin	TTCAGGCTGTTCTTTCTCTATATGC	GACTTCTGGACAACGGAATCTC

treatment, the tips of the shoots with three nodes were sprayed once until runoff. The untreated controls (HCT$^+$CKODA$^-$) were one-year-old shoots that were not sprayed with CKODA. At 7, 21, 35, 49, 63, 77, and 96 DAFB, 90 apical buds (18 buds per each tree) were randomly sampled. Apical buds were frozen immediately in liquid nitrogen and then stored at –80 °C until analysis of *MdTFL1* and *MdFT1* gene expression.

In the shade treatment, three randomly selected 19-year-old trees were covered with cheesecloth (50% shading) at 20 DAFB. The number of leaves per fruit was adjusted to 40 at 20 DAFB. This number of leaves per fruit is the usual fruit load not causing alternate bearing. One hundred twenty one-year-old shoots in each tree (360 apical buds in three trees), also randomly selected at 7 DAFB, were sprayed with 100 µM CKODA, like in the crop load experiment (Shade$^+$CKODA$^+$). At 7, 21, 35, 49, 63, and 77 DAFB, 60 apical buds (20 buds per tree) were randomly sampled for analysis of the expression of *MdTFL1* and *MdFT1* genes. The untreated controls (Shade$^+$CKODA$^-$) were one-year-old shoots that were not sprayed with CKODA.

To determine the proportion of flower bud formation in the cropload and shade experiments, 90 apical buds (three replications of 30 buds per tree) were randomly collected after defoliation. Each apical bud was longitudinally dissected, and the proportion of flower bud formation was determined with a stereomicroscope. Buds that apical meristems showed to be dome-shaped were counted as flower buds according to a previous report (Kittikorn et al., 2010). The proportion of flower bud formation was calculated by the number of flower buds in 90 apical buds.

Preparation of CKODA. CKODA ($C_{18}H_{31}ClO_5$) was formed by introducing a chlorine atom and a hydroxyl group at the C-15 olefin of KODA. KODA (32 mM) of 250 mL was mixed with 690 mL distilled water followed by the addition of 60 mL dilute aqueous solution [10% of commercial sodium hypochlorite solution (active chlorine 8.5% to 13.5%; Nacalai Tesque, Kyoto, Japan)] and vigorously mixed. The conversion rate of CKODA from KODA was more than 60%. CKODA was extracted by ethyl acetate and isolated by HPLC (NANOSPACE SI-1; Shiseido) using an ultraviolet detector at 210 nm with a Capcell Pak C_{18} column. The mobile phase was 50% acetonitrile in 0.05% trifluoroacetic acid, and the column temperature and flow rate were 30 °C and 1 mL·min^{-1}, respectively. The retention time of CKODA was 5.4 min. The product was identified with HPLC-MS/MS. The fragment ions of CKODA were *m/z* 363 [M-H]$^-$ and 345 [M-H-H_2O]$^-$.

RNA extraction, cDNA synthesis, and quantitative real-time RT-PCR. Total RNA from apical buds was extracted following the protocol mentioned in a previous report (Kittikorn et al., 2010). DNase treatment (5 units; Takara Bio, Otsu, Japan) at 37 °C for 20 min was applied to 2 µg of total RNA to eliminate any contaminating DNA in the samples. The reaction was stopped and then incubated at 70 °C for 10 min. The treated RNA was used as a template for cDNA synthesis. The cDNA was synthesized in a 20-µL reaction volume using ReverTraAce (Toyobo, Osaka, Japan) according to the manufacturer's instructions. RNA extraction and cDNA synthesis were repeated twice on each sampling date. The cDNA samples were diluted (1:10 v/v) and amplified using the gene-specific primers shown in Table 1. The quantitative reverse transcription–polymerase chain reaction (qRT-PCR) was performed using the SYBR master mix (KAPA SYBR® FAST qPCR kit; NIPPON Genetics, Tokyo, Japan) with ABI StepOnePlus (Applied Biosystems, Foster City, CA). The steps involved in amplification were performed as follows: an initial denaturation at 95 °C for 10 s followed by 40 cycles of amplification at 95 °C for 3 s and annealing at 60 °C for 32 s. The specificity of the amplification was verified on the basis of a melting curve obtained at the end of the PCR cycle. The relative expression level was normalized to that of the *actin* gene (Yooyongwech et al., 2008).

Statistical analysis. The SAS analysis of variance procedure (Version 8.02; SAS Institute, Cary, NC) was used to determine the treatment effects, and the mean separation was analyzed by Fisher's least significant difference ($P \leq 0.05$). The data are presented as the mean values of three replications ± SE.

Results

The proportion of flower bud formation of FTT was significantly higher than that of HCT (Table 2). A significant increase in endogenous KODA in FTT was observed at 63 and 103 DAFB, although there was no significant difference of KODA levels at 21 and 49 DAFB between HCT and FTT (Fig. 2). In HCT trees, endogenous KODA levels did not show significant changes from 21 to 103 DAFB.

The relative expression levels of *MdTFL1* in HCT$^+$CKODA$^+$ were significantly lower than those in HCT$^+$CKODA$^-$ at 21, 35, and 49 DAFB (Fig. 3A). In both treatments, the expression of

Table 2. Proportion of flower bud formation in apical buds of 'Fuji' apple treated with 15, 16-chloro, hydroxyl-9-hydroxy-10-oxo-12(Z)-octadecenoic acid (CKODA) under heavy-crop treatment (HCT) or shade.

Treatment[z]	Proportion of flower bud formation [mean ± SE (%)]
HCT$^+$CKODA$^-$	60.3 ± 0.58[y]
HCT$^+$CKODA$^+$	67.6 ± 0.60
FTT	81.3 ± 3.84
LSD$_{0.05}$	2.30
	22.0 ± 2.16
Shade$^+$CKODA$^-$	61.6 ± 0.74
Shade$^+$CKODA$^+$	6.35
LSD$_{0.05}$	

[z] HCT = number of leaves per fruit was adjusted to 20 at 20 d after full bloom (DAFB). Shade = trees covered with cheesecloth (50% shading) at 20 DAFB; the number of leaves per fruit was adjusted to 40 at 20 DAFB. FTT = flower thinning treatment, in which all flowers were removed at full bloom.

[y] The proportion of flower bud formation was calculated by the number of flower buds in 90 apical buds. Mean separation was analyzed by Fisher's least significant difference (LSD) ($P \leq 0.05$).

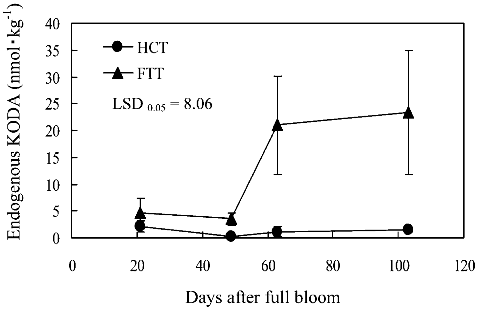

Fig. 2. Changes in endogenous 9, 10-ketol-octadecadienoic acid (KODA) concentrations in the apical buds of ‘Fuji’ apple subjected to the flower thinning treatment (FTT), in which all flowers were removed at full bloom and heavy-crop treatment (HCT), in which the number of leaves per fruit was adjusted to 20 at 20 d after full bloom. Data are mean ± SE of three replications.

MdTFL1 decreased sharply after 63 DAFB and leveled off at 77 DAFB in HCT$^+$CKODA$^+$ and HCT$^+$CKODA$^-$ at 98 DAFB. The expression patterns of *MdFT1* were similar in both CKODA-treated and -untreated buds under HCT conditions, although the times of the increases and decreases differed (Fig. 3B). That is, the expression of *MdFT1* in HCT$^+$CKODA$^-$ increased toward 49 DAFB and then decreased at 63 DAFB, whereas that in HCT$^+$CKODA$^+$ increased toward 35 DAFB and then decreased at 49 DAFB. The proportion of flower bud formation in HCT$^+$CKODA$^+$ was significantly higher than that in HCT$^+$CKODA$^-$ (Table 2).

In the shade treatment, the application of CKODA significantly increased the proportion of flower bud formation compared with that of untreated control (Table 2). The expression of *MdTFL1* genes at 7, 21, and 35 DAFB did not differ significantly between Shade$^+$CKODA$^+$ and Shade$^+$CKODA$^-$, but the expression of *MdTFL1* in Shade$^+$CKODA$^-$ was higher than that in Shade$^+$CKODA$^+$ at 49, 63, and 77 DAFB (Fig. 4A). In contrast, the expression in Shade$^+$CKODA$^+$ did not show much change.

In general, the patterns of change of *MdFT1* expression in both CKODA-treated and -untreated buds were similar under shade conditions (Fig. 4B). The *MdFT1* expressions increased toward 35 DAFB and then decreased at 49 DAFB. However, the expressions of *MdFT1* in Shade$^+$CKODA$^+$ were significantly higher than those in Shade$^+$CKODA$^-$.

Discussion

In a previous report, we reported the changes of endogenous KODA in apple buds with 15-hydroxypentadecanoic acid as the internal standard using a fluorescence detector in HPLC (Kittikorn et al., 2010). In the current study, endogenous KODA was analyzed using liquid chromatography–MS/MS for more accurate determination. The endogenous KODA concentrations in FTT were significantly higher than those of HCT. Furthermore, these concentrations increased significantly at 63 DAFB and before flower bud formation, as shown in a previous report (Kittikorn et al., 2010). This fact suggests that endogenous KODA may play a positive role in flower bud formation in apple. Either endogenous gibberellins (GAs) from the seeds or GA spray is known to inhibit flowering in apple (Ramirez et al., 2001, 2004). Therefore, it is possible that low endogenous KODA concentrations in the buds of HCT are associated with

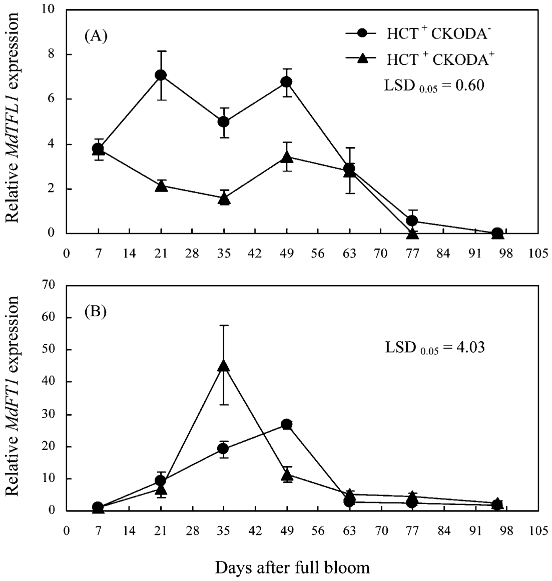

Fig. 3. Quantitative real-time reverse transcription–polymerase chain reaction (RT-PCR) analysis of (**A**) *MdTFL1* and (**B**) *MdFT1* in apical buds of ‘Fuji’ apple treated with 15, 16-chloro, hydroxyl-9-hydroxy-10-oxo-12(Z)-octadecenoic acid (CKODA) under heavy-crop treatment (HCT), in which the number of leaves per fruit was adjusted to 20 at 20 d after full bloom. The quantitative RT-PCR was performed using the SYBR master mix. The steps involved in amplification were performed as follows: an initial denaturation at 95 °C for 10 s followed by 40 cycles of amplification at 95 °C for 3 s and annealing at 60 °C for 32 s. The relative expression level was normalized to that of the *actin* gene. Data are mean ± SE of three replications.

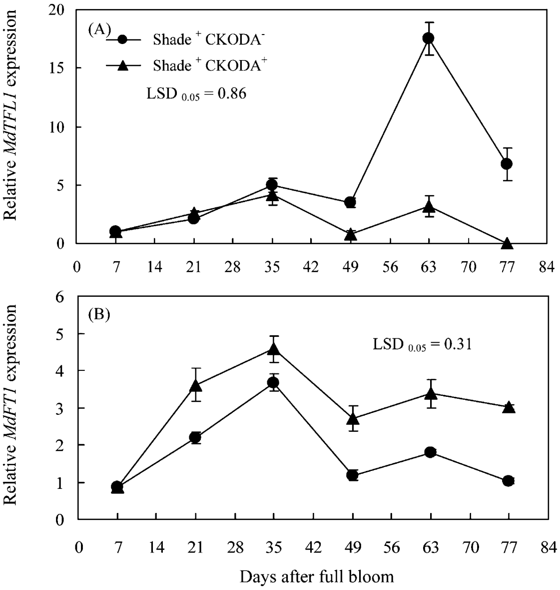

Fig. 4. Quantitative real-time reverse transcription–polymerase chain reaction (RT-PCR) analysis of (**A**) *MdTFL1* and (**B**) *MdFT1* in apical buds of ‘Fuji’ apple treated with 15, 16-chloro, hydroxyl-9-hydroxy-10-oxo-12(Z)-octadecenoic acid (CKODA) under shade, in which the trees were covered with cheesecloth (50% shading) at 20 d after full bloom (DAFB). The number of leaves per fruit was adjusted to 40 at 20 DAFB. The quantitative RT-PCR was performed using the SYBR master mix. The steps involved in amplification were performed as follows: an initial denaturation at 95 °C for 10 s followed by 40 cycles of amplification at 95 °C for 3 s and annealing at 60 °C for 32 s. The relative expression level was normalized to that of the *actin* gene. Data are mean ± SE of three replications.

high GA concentrations compared with those in FTT (Kittikorn et al., 2010). However, KODA application to the buds did not influence endogenous GA concentrations in the buds (Kittikorn et al., 2011). Therefore, the relationship between auxin and cytokinin, which influence flower bud formation (Sanyal and Bangerth, 1998), and KODA should also be examined. In addition, the origin of endogenous KODA in the buds of apple requires further investigation.

It has been shown that *FT* and *TFL1* homologs are important in regulating the flowering time and maintaining the inflorescence meristem in several plant species (Hanke et al., 2007). A homolog of the *FT* and *TFL1* gene family has been isolated and characterized in fruit trees such as apple (Kotoda and Wada, 2005; Kotoda et al., 2006), grape (Carmona et al., 2007), sweet orange [*Citrus sinensis* L. Osbeck (Pillitteri et al., 2004)], and pear and quince [*Pyrus pyrifolia* Nakai and *Cydonia oblonga* Mill. (Esumi et al., 2005)]. *MdTFL1* gene in apple, which is a *TFL1*-like gene, functions equivalently to *TFL1* expressed constitutively in transgenic arabidopsis plants, suggesting that it has the potential to complement the *TFL1* function (Kotoda et al., 2006). It could function redundantly as a flowering repressor and a regulator of vegetative meristem identity because *MdTFL1* is detected strongly in vegetative tissues (Mimida et al., 2009). Our previous report (Kittikorn et al., 2011) showed that the accumulation of *MdTFL1* mRNA transcript levels was found in buds under both heavy-crop and shade treatments that related to the decrease of flower bud formation. The proportion of flower bud formation in apical buds of apple increased after 70 DAFB (Kittikorn et al., 2010). In our study, CKODA application in HCT decreased *MdTFL1* mRNA transcript levels at 21, 35, and 49 DAFB, although the expression of *MdTFL1* in HCT$^+$CKODA$^-$ decreased sharply after 63 DAFB. It has been shown that *MdTFL1* mRNA was expressed strongly in June, which is 7 weeks after full bloom, which occurs 1 to 2 weeks after the initiation of floral bud formation; from June, the expression decreased gradually toward late July (Kotoda and Wada, 2005). In our study, the proportion of flower bud formation in Shade$^+$CKODA$^-$ was lower than that in HCT$^+$CKODA$^-$. In addition, the expression of *MdTFL1* in Shade$^+$CKODA$^-$ was higher than that in HCT$^+$CKODA$^-$ at 63 DAFB. Although the *MdTFL1* expression in Shade$^+$CKODA$^-$ decreased at 77 DAFB, the expression level was significantly higher than that of Shade$^+$CKODA$^+$. These results support that the induction of flower bud formation and floral development may be coincident with the interruption of *MdTFL1* expression (Kotoda and Wada, 2005; Mimida et al., 2009). Furthermore, *MdTFL1* expression in our study shows that low light intensity may have a more negative effect on flower bud formation than a heavy cropload.

Contrary to the functional role of *TFL1*, *FT* promoted flowering by altering the expression of other endogenous genes (Esumi et al., 2007; Kardailsky et al., 1999). The mobile FT protein has been identified as a main component of the florigen or flowering hormone in arabidopsis, tomato, and rice (Lifschitz et al., 2006; Turck et al., 2008; Zeevaart, 2008). In apple, *MdFT1* and *MdFT2* genes have been isolated and their functions clarified (Kotoda and Wada, 2005; Kotoda et al., 2010). A transgenic experiment revealed that both *MdFT1* and *MdFT2* had the potential to function as floral promoters in apple. However, the transcriptional activation of *MdFT1* is more closely associated with the regulation of flower induction in apple than that of *MdFT2*. Therefore, we examined the transcription level of *MdFT1*. In our study, the expression of *MdFT1* in HCT$^+$CKODA$^+$ reached a peak earlier and higher than in HCT$^+$CKODA$^-$. It has been reported that the increase of *MdFT1* (Hättasch et al., 2009; Kotoda et al., 2010) and *AFL1* and *AFL2* transcription levels (apple orthologs of *LFY*) (Wada et al., 2002) in the apical meristems of one-year-old apple shoots began 1 to 2 weeks earlier than flower initiation. In our study, *MdFT1* expression levels in HCT$^+$CKODA$^-$ were higher than those in Shade$^+$CKODA$^-$ at 21 to 49 DAFB. The expressions of *MdFT1* were increased earlier by CKODA application in both HCT and shade treatment. The increase of the proportion of flower bud formation at 70 DAFB (Kittikorn et al., 2010), which did not occur after the increase of *MdFT1* but did occur after the decrease of *MdTFL1* expression, suggests that the role of *MdTFL1* may be downstream of *MdFT1*. It was demonstrated that the dynamic state of *MdFT1* and *MdTFL1* genes observed in our study may be associated with flower bud formation.

In summary, endogenous KODA in the buds of trees in which the proportion of flower bud formation was high increased before the initiation of flower bud formation. In heavy-crop and shade-treated trees, the application of CKODA, a derivative of KODA, increased the proportion of flower bud formation and the expression of *MdFT1* but decreased the expression of *MdTFL1*.

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