BIOLOGIA PLANTARUM 62 (1): 194-199, 2018 DOI: 10.1007/s10535-017-0743-6

BRIEF COMMUNICATION

Photoperiod and ethylene-dependent expression of gibberellin biosynthesis gene *InEKO1* during flower induction of *Ipomoea nil*

K. MARCINIAK[1,2]*, E. WILMOWICZ[1,2], A. KUĆKO[1], and J. KOPCEWICZ[1]

Chair of Plant Physiology and Biotechnology, Nicolaus Copernicus University, PL-87100 Toruń, Poland[1]
Centre for Modern Interdisciplinary Technologies, Nicolaus Copernicus University, PL-87100 Toruń, Poland[2]

Abstract

Ent-kaurene oxidase (EKO) catalyze three sequential oxidations in the early steps of gibberellin biosynthesis pathway. In this research, a cDNA sequence of *InEKO1* gene in the model short-day plant *Ipomoea nil* was identified. Our studies revealed that inductive conditions for flowering caused an increase in the transcriptional activity of the examined gene in the cotyledons – the main organs for the perception of the photoperiodic stimulus. In contrast, in the second half of the 16 h long inductive night and after that, a decreased amount of *InEKO1* mRNA in the apexes was detected. What is more, ethylene, the key inhibitor of flower induction in *I. nil,* elevated the *InEKO1* expression exclusively in the cotyledons between 10 and 14 h of the inductive night.

Additional key words: *ent*-kaurene oxidase, flowering, phytohormones, short-day plant.

Gibberellins (GAs) are a family of tetracyclic, diterpenoid plant hormones controlling, *e.g.*, germination, stem elongation, and flowering (Yamaguchi 2008, Liu *et al*. 2017). One of the early steps in the GA biosynthesis is the conversion of *ent*-kauren to *ent*-kaurenoic acid through *ent*-kaurenol and *ent*-kaurenal *via* three oxidation steps, which are catalyzed by membrane-associated cytochrome P_{450} monooxygenase – *ent*-kaurene oxidase (EKO) (Ko *et al.* 2008, Marciniak *et al.* 2012b). Research conducted in several plants species have shown that the transcriptional activity of genes coding for enzymes involved in GA metabolism is regulated by photoperiod and phytohormones (Wu *et al.* 1996, Xu *et al.* 1997, Calvo *et al.* 2004, Marciniak *et al.* 2012a). This provides an appropriate GA content in the particular organs at the right time determining the correct development of plant.

In the last three decades, most molecular studies on the role of GAs in the transition from the vegetative to generative phase were carried out on *Arabidopsis thaliana*, which is the classical example of a long-day (LD) plant (Wilson *et al.* 1992, Blazquez *et al.* 1998, Cheng *et al.* 2004). It has been established that GAs control the spatial expression of various floral regulatory genes in a day-length-specific manner in both cotyledons and apexes (Galvao *et al*. 2012). Under inductive conditions, these phytohormones increase the levels of *FLOWERING LOCUS T* (*FT*) and *TWIN SISTER OF FT* (*TSF*) mRNAs, encoding a systemic signal inducing flowering transported from the leaves to the apex. Additionally, GAs promote transcript accumulation of *SQUAMOSA PROMOTER BINDING PROTEIN-LIKE* (*SPL*) gene (Galvao *et al*. 2012, Porri *et al*. 2012). On the other hand, in non-inductive conditions, GAs enhance the transcriptional activity of *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* (*SOC1*) (Moon *et al*. 2003) and *LEAFY* (*LFY*) (Blazquez *et al*. 1998) exclusively at the apex. Interestingly, in another LD plant *Lolium temulentum*, GAs have not affected the *FT* expression, both in LD as well as in short-day (SD) conditions, suggesting that these factors function independently in the flower induction. What is more, GAs produced in leaves under inductive conditions are mobile signal triggering flowering at the apex (King *et al*. 2006).

The genetic and molecular knowledge concerning photoperiod- and GA-controlled flowering in SD plants

Submitted 19 December 2016, *last revision* 15 March 2017, *accepted* 20 March 2017.
Abbreviations: EKO - *ent*-kaurene oxidase; ET - ethylene; GAs - gibberellins; LD - long day; SD - short day.
Acknowledgements: The work was supported by the National Science Centre (Poland) grant No. N N303 811240 and the project entitled "Scholarships for doctoral 2008/2009 – ZPORR" grant No. SPS. IV-3040-UE/619/2009.
* Author for correspondence; e-mail: marciniak@umk.pl

still remains poorly understood. It has been shown that the genomes of *Oryza sativa* and *Ipomoea nil* contain genes of the photoperiodic induction pathway identified in *A. thaliana*, *e.g.*, *CONSTANS* (*CO*) and *FT*; however, the regulation of their expression is varied (Hayama and Coupland 2004, Hayama *et al*. 2007). *In vitro* studies performed in another SD plant *Chenopodium rubrum* indicate that gibberellic acid (GA_3) stimulates flowering under continuous darkness (Mitrović *et al*. 2003).

The critical regulator of flower induction is also ethylene (ET) (Ogawara *et al.* 2003, Kęsy *et al*. 2008). Many results indicate that ET and GAs can act synergistically, whereas in other processes, they can be antagonistic (Raab and Koning 1987, Achard *et al*. 2003, Mignoli *et al*. 2016). It was presented, that ET inhibits the expression of GA metabolism gene, *e.g.*, *FsGA20ox1* in seeds of *Fagus sylvatica* (Calvo *et al*. 2004). ET reduces also bioactive GAs content, thus promoting the accumulation of DELLAs repressor proteins, and consequently delays floral transition in *A. thaliana* (Achard *et al*. 2007).

To understand the fundamental mechanism controlling plant sexual reproduction, research conducted on different species is of a great importance. Physiological and biochemical investigations revealed that *I. nil* is a perfect model for study of the photoperiodic flower induction, because exposing cotyledons of 5-d-old seedlings to only a single 16-h long night is sufficient for induction (Ogawa 1981). Therefore, in this paper, we identified and analyzed the full-length cDNA of the *InEKO1* gene in *I. nil*. We examined the *InEKO1* mRNA content in the cotyledons and apexes under inductive and non-inductive conditions as well as after treatment with ET under inductive conditions.

The seeds of *Ipomoea nil* (L.) Roth. cv. Violet were obtained from the *Marutane Seed Company* (Kyoto, Japan) and prepared and sown according to Wilmowicz *et al.* (2011). Plants were cultivated for 5 d in a growth chamber at a temperature of 26 ± 1 °C, a relative humidity of 65 %, an irradiance of 130 μmol m^{-2} s^{-1} (cool white fluorescent tubes, *Polam*, Warsaw, Poland) under non-inductive conditions (16 h light/8 h darkness, LD) (Fig. 1 Suppl.). Part of them were exposed to inductive conditions (16 h darkness/8 h light, SD). Then half of plants cultivated under SD conditions were additionally treated with gaseous ET. For phytohormone application, the plants in pots were enclosed in 9-dm^3 glass containers and ET at concentration of 0.1 cm^3 dm^{-3} was put *via* a syringe with septum. The injection was repeated every 2 h during the inductive night (at 4, 6, 8, 10, and 12 h) with ventilation before each treatment. After 14 h all applications stopped. The excised cotyledons and apexes were immediately frozen in liquid nitrogen and stored at -80 °C before RNA isolation. All the procedures performed during the dark period were carried out under safe dim green light. For the physiological study, fifteen plants from all experimental variants (SD, LD, SD+ET) were kept growing for 5 - 7 weeks to determine the percentage of formed vegetative and floral buds. Each experiment was repeated at least three times, and the results were presented as means ± SEs.

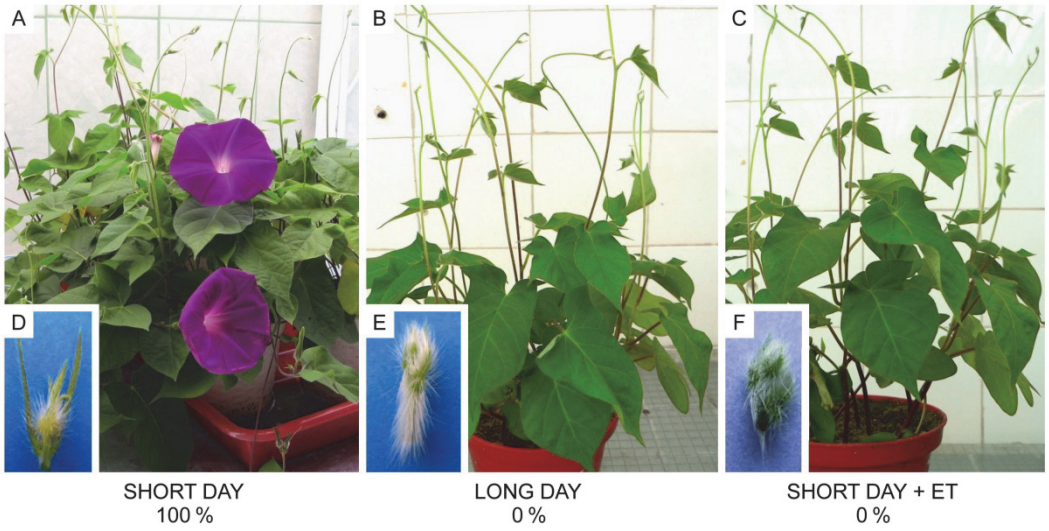

Fig. 1. The influence of photoperiod and ethylene (ET) on flowering of *Ipomoea nil*. The plants were subjected to a 16-h inductive night (SHORT DAY) (*A*); an 8-h non-inductive night (LONG DAY) (*B*); a 16-h inductive night with an additional ET application (SHORT DAY + ET) (*C*). Enlarged images of generative (*D*) and vegetative (*E*, *F*) buds are also presented. Flowering was 100 % in *A* and 0 % in *B* and *C*.

In order to identify *InEKO1* cDNA, the cotyledons (1 g) were ground into powder using a mortar and pestle, and placed in a chilled Eppendorf tube. For total RNA isolation, the column method (*GeneMATRIX* universal RNA purification kit; *EURx*, Gdańsk, Poland) was used and the genomic DNA was removed from the sample by deoxyribonuclease I (*Fermentas*, St. Leon-Rot, Germany). In both procedures, the manufacturer's

recommendations were followed. Reverse transcription was performed using oligo(dT)$_{19}$ primers, *RevertAid*^{TM} *M-MuLV* reverse transcriptase (*Fermentas*) and 1 μg of total RNA. The degenerate primers (Table 1 Suppl.) were designed for highly conserved cDNA sequences. The touchdown PCR reactions were performed in the *T3* thermocycler (*Biometra*, Göttingen, Germany) using 1.25 U *Perpetual Taq* DNA *Polymerase*$^{HOT\ START}$ (*EURx*), 0.1 μg of first-strand cDNA, 1× buffer B containing 15 mM Mg^{2+}, 0.2 mM dNTP mix, 1.5 mM Mg^{2+}, 1 μM primer solution, and deionized water to a final volume of 50 mm^3. A 334 bp amplified cDNA fragment was isolated, purified from an agarose gel (*GeneMATRIX Agarose Out* DNA purification kit, *EURx*) and ligated into pCRII-TOPO vector (*TOPO TA* cloning kit, *Invitrogen*, Carlsbad, USA). Next, the plasmids were transferred into chemically competent *One Shot Mach1-T1 Escherichia coli* cells which were plated onto Petri dishes containing *S-Gal/LB Agar Blend* (*Sigma*, St. Louis, USA) and 50 μg cm^{-3} ampicillin (*ICN*). White colonies were selected and cultured in liquid 2× Lourain Brot medium containing 50 μg cm^{-3} ampicillin. The plasmid DNA was isolated (*GeneMATRIX Plasmid Miniprep* DNA purification kit, *EURx*) and sequenced by *Genomed* (Warsaw, Poland). A full-length cDNA was obtained using designed primers (Table 1 Suppl.) and the *SMARTer™ RACE* cDNA amplification kit (*Clontech*, Palo Alto, USA) or *FirstChoice RLM-RACE* kit (*Ambion*, Austin, USA) in the 5' and 3' RACE-PCR reactions, respectively, according to the manufacturer's recommendations. The reaction products were isolated, purified from the gel, cloned, and sequenced in the same manner as previously described.

Total RNA for transcriptional activity studies was isolated from the cotyledons and apexes using the column method (*GeneMATRIX* universal RNA purification kit) and *TRI-Reagent* (*Sigma*), respectively. Total RNA (1 μg) primed with anchored oligo(dT)$_{18}$ primers was used for first-strand synthesis with *Transcriptor High Fidelity* cDNA synthesis kit (*Roche*, Mannheim, Germany). For genes quantification, real-time quantitative PCR reactions with the specific primers and universal probe library (*UPL*) hydrolysis probes (Table 1 Suppl.) were used. The *actin* gene (*InACT4*) was selected as a reference endogenous control for normalization (Glazińska *et al.* 2014, Wilmowicz *et al*. 2016). The expression of the studied genes was determined using the *LightCycler 2.0* system and a commercial qPCR reaction kit (*LightCycler TaqMan Master*, *Roche*) following the manufacturer's instructions. A negative control (cDNA-free) for each qPCR reaction was used. The reactions were carried out in glass capillaries (volume 20 mm^3) as follows: 95 °C for 600 s; 45 cycles of 95 °C for 10 s, 58 °C for 15 s and 72 °C for 1 s; and 40 °C for 30 s. The results were analyzed using *LightCycler* software *4.1* (*Roche*). The relative expression of each sample was calculated by dividing the expression of *InEKO1* gene by that of *InACT4*. The reaction efficiency (>1.8) was obtained on the basis of relative quantification calculated using standard curves from serial dilutions of cDNAs. For each cDNA template, at least three independent technical replications were performed. The Student's *t*-test was used to determine the significant differences compared to the control. *SigmaPlot 2001 v. 7.0* was used for statistical analysis and charts drawing.

The results of research conducted in LD plants, as well as in neutral plants indicate that EKO protein play an essential role in the early stages of GA biosynthesis (Helliwell *et al.* 1998, 2000, Li *et al.* 2010, Morrone *et al.* 2010). However, much less is known about this phenomenon in SD plants (Ko *et al.* 2008). In this paper we obtained a full-length cDNA of *InEKO1* gene (NCBI, GenBank acc. No. HQ169128, Fig. 2 Suppl.) by using degenerate primers and 5'-3' RACE-PCR reactions (Fig. 2 Suppl.). The studies performed by Ko *et al.* (2008) in SD plant *Oryza sativa* indicate, that mutations in *EKO* gene (*OsKOL2*) cause severe GA-deficiency and dwarfism, confirming its involvement in GA biosynthesis. The comparison between deduced amino acid sequences of InEKO1 (*NCBI*, GenPept No. ADT64304) and EKO proteins from other plant species (Figs. 3 Suppl. and 4 Suppl.) revealed the closest evolutionary relationship with polypeptides from *Solanales* order (Fig. 3 Suppl.). It was found, that InEKO1 contained all motifs and catalytic domains characteristic for the superfamily of cytochrome P_{450} monooxygenases and conserved among EKO proteins, which suggest that encodes functional enzyme (Fig. 2 Suppl.) (Kalb and Loper 1988, Villa-Ruano *et al.* 2010).

Radiation and phytohormones are the fundamental factors affecting the flowering time (for review see Mutasa-Göttgens and Hedden 2009). Additionally, there are numerous reports claiming that photoperiod controls the transcriptional activity of the genes coding for enzymes that catalyze the late stages of GA biosynthesis (*GA20ox*, *GA3ox*) or inactivation (*GA2ox*) (Wu *et al*. 1996, Xu *et al*. 1997, Sakamoto *et al*. 2001, King *et al*. 2006, Lee and Zeevaart 2007). However, only a few papers concerning the effect of that factor on the regulation of early stages of GA biosynthesis have been published (Zeevaart and Gage 1993, Yamaguchi *et al.* 1998). Therefore, we performed a thorough analysis of *InEKO1* expression in the cotyledons and apexes of 5-d-old *I. nil* seedlings, from plants cultivated under SD or LD conditions and during inductive night with additional ET treatment.

The obtained results revealed that the *InEKO1* expression is photoperiod-dependent both in the cotyledons, as well as in apexes (Fig. 2). When we exposed the plants grown under non-inductive night (8 h) to irradiance of 130 μmol m^{-2} s^{-1} we observed strong decrease in the amount of *InEKO1* transcripts in the cotyledons (Fig. 2*A*). It was almost four times lower in comparison to the beginning of the night. Simultaneously, these plants did not flower (Fig. 1*B*). In turn, in the second half of the 16-h inductive night the *InEKO1* expression was in average 2 - 3 times higher in comparison to LD plants in every time point (Fig. 2*A*).

However, transferring the plants from 16-h inductive night to irradiance of 130 μmol m^{-2} s^{-1} caused strong and transient accumulation of *InEKO1* mRNA (18 h). All plants cultivated under inductive conditions formed flowers (Fig. 1*A*).

Our analyses also indicate that *InEKO1* was more expressed in every examined variant in the apexes than in the cotyledons (Fig. 2). Interesting is the fact that the expression pattern was different in these organs (Fig. 2*A*,*B*). The cDNA content in the apexes was relatively stable and lower in the SD conditions than in the LD conditions, even more during light phase (18 - 24 h) (Fig. 2*B*). The exposition of of plants cultivated under LD conditions to irradiance (at 8 h) resulted in a gradual accumulation of *InEKO1* transcripts, which reached the maximum value at 18 h and consequently decreased in subsequent hours (Fig. 2*B*).

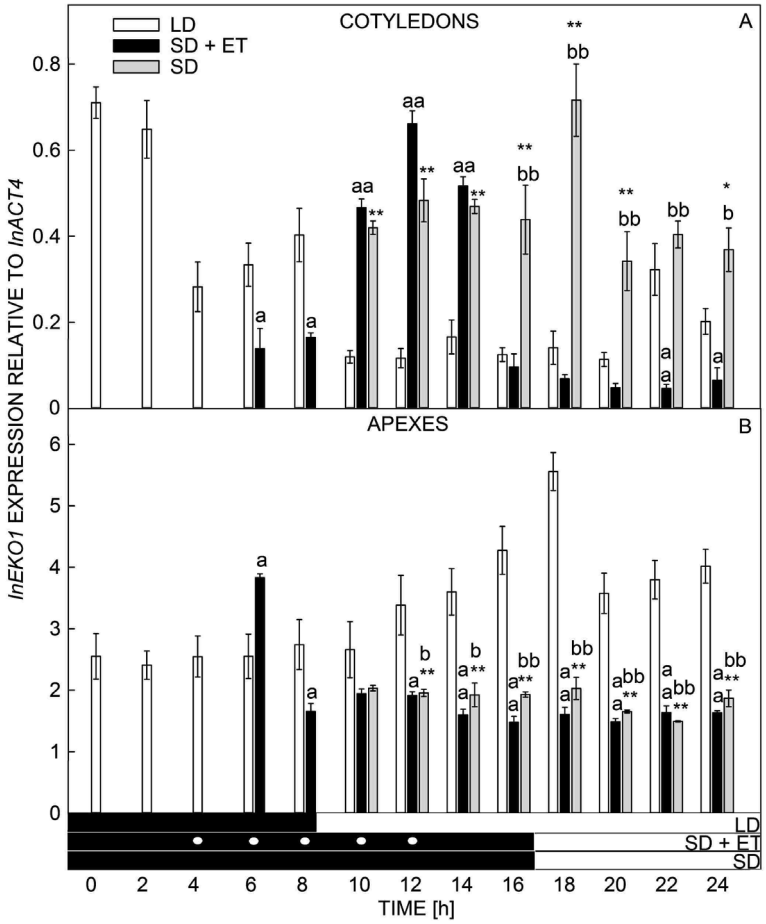

Fig. 2. *InEKO1* gene expression (relative to *InACT4* reference gene) in the cotyledons (*A*) and apexes (*B*) of 5-d-old *Ipomoea nil* seedlings grown under various photoperiodic conditions and after ethylene (ET) application. The horizontal, *black lines* under the x-axis demonstrate duration of the dark period. SD - short day, LD - long day. ET treatment (0.1 cm^3 dm^{-3}) was indicated by *white spots*. All data are the results of three biological and tree technical replications (n = 9). Means ± SEs. Significant differences in *InEKO1* expression changes: *1*) in plants growing under SD conditions in comparison to plants growing under LD conditions are indicated as ** - $P < 0.01$ and * - $P < 0.05$; *2*) in plants treated with ET (SD + ET) in comparison to plants growing under LD conditions are indicated as ^{aa} - $P < 0.01$ and ^{a} - $P < 0.05$; *3*) in plants treated SD + ET in comparison to plants growing under SD conditions are indicated as ^{bb} - $P < 0.01$ and ^{b} - $P < 0.05$.

An analysis of *InEKO1* expression under inductive and non-inductive conditions revealed regulation by photoperiod, which might suggest that its promoter contains sequences regulated by factors involved in the light signal transduction pathway. The functioning of a similar mechanism during the light-regulated germination of *A. thaliana* seeds was observed (Kim *et al*. 2008, Gabriele *et al*. 2010). Phytochrome, photoreceptor responsible for the perception of light stimuli, regulates the amount of the PHYTOCHROME INTERACTING FACTOR 3-LIKE 5 (PIL5). This protein, affects the activity of the SOMNUS (SOM) and DOF AFFECTING GERMINATION 1 (DAG1) transcriptional factors, which in turn control the expression of *GA3ox2*/*GA2ox2* and *AtGA3ox1*, respectively (Kim *et al*. 2008, Gabriele *et al*. 2010). *InEKO1* expression pattern in the cotyledons and apexes during flower induction also suggests its involvement in perceiving the photoperiodic stimulus by

cotyledons. A significant participation of the AtEKO/GA3 enzyme in proper leaf development was already confirmed in *A. thaliana*. A single *ga3* mutant demonstrates a severe dwarf phenotype with small rosette leaves (Helliwell *et al*. 1998).

Ethylene, which inhibits flowering of *I. nil* (Fig. 1*C*), stimulates the expression of *InEKO1* gene in the cotyledons, but only in the second half of the inductive night (10 - 14 h) (Fig. 2*A*). On the contrary, ET did not cause any effect in the apexes (Fig. 2*B*). Kęsy *et al.* (2008) revealed that flowering of *I. nil* depends on a low content of ET in the second half of the inductive night. To date, there are no published data concerning the role of ET in the regulation of *EKO* expression. However, the expression of most genes from the cytochrome P_{450} monooxygenase genes family is affected by phyto-hormones, *e.g*. salicylic acid, jasmonic acid, abscisic acid, as well as by ET (Narusaka *et al.* 2004). Nevertless, Zeevart and Gage (1993) proved that in *Spinacia oleracea* and *Agrostemma githago* the content of *ent*-kaurene, which is a substrate for *ent*-kaurene oxidase, is higher in the mature leaves under inductive conditions in comparison to non-inductive ones.

In conclusion, our study confirmed that *InEKO1* demonstrates a high homology to *EKO* genes from other plant species. Moreover, the obtained results are the first determining the effects of photoperiod and ET on the expression of gene coding for *ent*-kaurene oxidase in model SD plant, *I. nil*. Summarizing, the early stages of GA biosynthesis in the flower induction of *I. nil* are light- and ET-regulated. This study provides a promising advances in our understanding of regulation of GA biosynthesis by these factors contributing to the progress in knowledge concerning flower induction in SD plants.

References

Achard, P., Baghour, M., Chapple, A., Hedden, P., Van der Straeten, D., Genschik, P., Moritz, T., Harberd, N.P.: The plant stress hormone ethylene controls floral transition *via* DELLA-dependent regulation of floral meristem-identity genes. - PNAS **104**: 6484-6489, 2007.

Achard, P., Vriezen, W.H., Van der Straeten, D., Harberd, N.: Ethylene regulates *Arabidopsis* development via the modulation of DELLA protein growth repressor function. - Plant Cell **15**: 2816-2825, 2003.

Blazquez, M.A., Green, R., Nilsson, O., Sussman, M.R., Weigel, D.: Gibberellins promote flowering of *Arabidopsis* by activating the *LEAFY* promoter. - Plant Cell **10**: 791-800, 1998.

Calvo, A.P., Nicolas, C., Nicolas, G., Rodriguez, D. Evidence of a cross-talk regulation of a *GA 20-oxidase* (*FsGA20ox1*) by gibberellins and ethylene during the breaking of dormancy in *Fagus sylvatica* seeds. - Physiol. Plant **120**: 623-630, 2004.

Cheng, H., Qin, L., Lee, S., Fu, X., Richards, D.E., Cao, D., Luo, D., Harberd, N.P., Peng, J.: Gibberellin regulates *Arabidopsis* floral development *via* suppression of DELLA protein function. - Development **131**: 1055-1064, 2004.

Gabriele, S., Rizza, A., Martone, J., Circelli, P., Costantino, P., Vittorioso, P.: The Dof protein DAG1 mediates PIL5 activity on seed germination by negatively regulating GA biosynthetic gene *AtGA3ox1*. - Plant J. **61**: 312-323, 2010.

Galvao, V.C., Horrer, D., Kuttner, F., Schmid M.: Spatial control of flowering by DELLA proteins in *Arabidopsis thaliana*. - Development **139**: 4072-4082, 2012.

Glazińska, P., Wojciechowski, W., Wilmowicz, E., Zienkiewicz, A., Frankowski, K., Kopcewicz, J.: The involvement of *InMIR167* in the regulation of expression of its target gene *InARF8*, and their participation in the vegetative and generative development of *Ipomoea nil* plants. - J. Plant Physiol. **171**: 225-234, 2014.

Hayama, R., Agashe, B., Luley, E., King, R., Coupland, G.: A circadian rhythm set by dusk determines the expression of *FT* homologs and the short-day photoperiodic flowering response in *Pharbitis*. - Plant Cell **19**: 2988-3000, 2007.

Hayama, R., Coupland, G.: The molecular basis of diversity in the photoperiodic flowering responses of *Arabidopsis* and rice. - Plant Physiol. **135**: 677-684, 2004.

Helliwell, C.A., Olive, M.R., Gebbie, L., Forster, R., Peacock, W.J., Dennis, E.S.: Isolation of an *ent*-kaurene oxidase cDNA from *Cucurbita maxima*. - Aust. J. Plant Physiol. **27**: 1141-1149, 2000.

Helliwell, C.A., Sheldon, C.C., Olive, M.R., Walker, A.R., Zeevaart, J.A.D., Peacock, W.J., Dennis, E.S.: Cloning of the *Arabidopsis ent*-kaurene oxidase gene *GA3*. - PNAS **95**: 9019-9024, 1998.

Kalb, V.F., Loper, J.C.: Proteins from eight eukaryotic cytochrome P-450 families share a segmented region of sequence similarity. - PNAS **85**: 7221-7225, 1988.

Kęsy, J., Maciejewska, B., Sowa, M., Szumilak, M., Kawałowski, K., Borzuchowska, M., Kopcewicz, J.: Ethylene and IAA interactions in the inhibition of photoperiodic flower induction of *Pharbitis nil*. - Plant Growth Regul. **55**: 43-50, 2008.

Kim, D.H., Yamaguchi, S., Lim, S., Oh, E., Park, J., Hanada, A., Kamiya, Y., Choi, G.: SOMNUS, a CCCH-type zinc finger protein in *Arabidopsis*, negatively regulates light-dependent seed germination downstream of PIL5. - Plant Cell **20**: 1260-1277, 2008.

King, RW., Moritz, T., Evans, L.T., Martin, J., Andersen, C.H., Blundell, C., Kardailsky, I., Chandler, P.M.: Regulation of flowering in the long-day grass *Lolium temulentum* by gibberellins and the *FLOWERING LOCUS T* gene. - Plant Physiol. **141**: 498-507, 2006.

Ko, K.W., Lin, F., Katsumata, T., Sugai, Y., Miyazaki, S., Kawaide, H., Okada, K., Nojiri, H., Yamane, H.: Functional identification of a rice *ent*-kaurene oxidase, *OsKO2*, using the *Pichia pastoris* expression system. - Biosci. Biotech. Biochem. **72**: 3285-3288, 2008.

Lee, D.J., Zeevaart, J.A.D.: Regulation of *gibberellin 20-oxidase1* expression in spinach by photoperiod. - Planta **226**: 35-44, 2007.

Li, J., Tian, Y., Wang, C., Tian, W., Song, W., Yin, H.: Cloning and bioinformatics analysis of *ent*-kaurene oxidase gene *PpKO* in pear (*Pyrus pyrifolia* Nakai). - Acta hort. sin. **37**: 1575-1582, 2010.

Liu, K., Feng. S., Jiang, Y., Li, H., Huang, S., Liu, J., Yuan, C.: Identification and expression analysis of seven MADS-box genes from *Annona squamosa*. - Biol. Plant. **61**: 24-34, 2017.

Marciniak, K., Grzegorzewska, W., Kęsy, J., Szmidt-Jaworska, A., Tretyn, A., Kopcewicz, J.: Regulation of gibberellins metabolism in plants. - Kosmos **61**: 213-232, 2012a.

Marciniak, K., Kęsy, J., Tretyn, A., Kopcewicz, J.: Gibberellins - structure, biosynthesis and deactivation in plants. - Post. Biochem. **58**: 14-25, 2012b.

Mignolli, F., Rojas, G.B., Vidoz, M.L.: Supraoptimal ethylene acts antagonistically with exogenous gibberellins during *Solanum lycopersicum* (Solanaceae) hypocotyl growth. - Bol. Soc. Argent. Bot. **51**: 235-242, 2016.

Mitrović, A., Živanović, B., Ćulafić, L.: Effect of darkness on growth and flowering of *Chenopodium rubrum* and *C. murale* plants *in vitro*. - Biol. Plant. **46**: 471-474, 2003.

Moon, J., Suh, S.S., Lee, H., Choi, K.R., Hong, C.B., Paek, N.C., Kim, S.G., Lee, I.: The *SOC1* MADS-box gene integrates vernalization and gibberellin signals for flowering in *Arabidopsis*. - Plant J. **35**: 613-623, 2003.

Morrone, D., Chen, X., Coates, R.M., Peters, R.J.: Characterization of the kaurene oxidase CYP701A3, a multifunctional cytochrome P450 from gibberellin biosynthesis. - Biochem. J. **431**: 337-344, 2010.

Mutasa-Göttgens, E., Hedden, P.: Gibberellin as a factor in floral regulatory networks. - J. exp. Bot. **60**: 1979-1989, 2009.

Narusaka, Y., Narusaka, M., Seki, M., Umezawa, T., Ishida, J., Nakajima, M., Enju, A., Shinozaki, K.: Crosstalk in the responses to abiotic and biotic stresses in *Arabidopsis*: analysis of gene expression in cytochrome P450 gene superfamily by cDNA microarray. - Plant mol. Biol. **55**: 327-342, 2004.

Ogawa, Y., King, R.W.: The inhibition of flowering by non-induced cotyledons of *Pharbitis nil*. - Plant Cell Physiol. **31**: 129-135, 1990.

Ogawara, T., Higashi, K., Kamada, H., Ezura, H.: Ethylene advances the transition from vegetative growth to flowering in *Arabidopsis thaliana*. - J. Plant Physiol. **160**: 1335-1340, 2003.

Porri, A., Torti, S., Romera-Branchat, M., Coupland, G.: Spatially distinct regulatory roles for gibberellins in the promotion of flowering of *Arabidopsis* under long photoperiods. - Development **139**: 2198-2209, 2012.

Raab, M., Koning, R: Interacting roles of gibberellin and ethylene in corolla expansion of *Ipomoea nil* (Convolvulaceae). - Amer. J. Bot. **74**: 921-927, 1987.

Sakamoto, T., Kobayashi, M., Itoh, H., Tagiri, A., Kayano, T., Tanaka, H., Iwahori, S., Matsuoka, M.: Expression of a *gibberellin 2-oxidase* gene around the shoot apex is related to phase transition in rice. - Plant Physiol. **125**: 1508-1516, 2001.

Villa-Ruano, N., Betancourt-Jimenez, M.G., Lozoya-Gloria, E.: cDNA isolation and gene expression of kaurene oxidase from *Montanoa tomentosa* (zoapatle). - Rev. Latinoam. Quim. **38**: 21-28, 2010.

Wilmowicz, E., Frankowski, K., Glazińska P., Kesy, J., Wojciechowski, W., Kopcewicz, J.: Cross talk between phytohormones in the regulation of flower induction in *Pharbitis nil*. - Biol. Plant. **55**: 757-760, 2011.

Wilmowicz, E., Kućko, A., Frankowski, K., Świdziński, M., Marciniak, K., Kopcewicz, J.: Methyl jasmonate-dependent senescence of cotyledons in *Ipomoea nil*. - Acta Physiol. Plant. **38**: 222, 2016.

Wilson, R.N., Heckman, J.W., Somerville, C.R.: Gibberellin is required for flowering in *Arabidopsis thaliana* under short days. - Plant Physiol. **100**: 403-408, 1992.

Wu, K., Li, L., Gage, D.A., Zeevaart, J.A.: Molecular cloning and photoperiod-regulated expression of gibberellin 20-oxidase from the long-day plant spinach. - Plant Physiol. **110**: 547-554, 1996.

Xu, Y.L., Gage, D.A., Zeevaart, J.A.: Gibberellins and stem growth in *Arabidopsis thaliana*. Effects of photoperiod on expression of the *GA4* and *GA5* loci. - Plant Physiol. **114**: 1471-1476, 1997.

Yamaguchi, S.: Gibberellin metabolism and its regulation. - Annu. Rev. Plant Biol. **59**: 225-251, 2008.

Yamaguchi, S., Sun, T.-P., Kawaide, H., Kamiya, Y.: The *GA2* locus of *Arabidopsis thaliana* encodes *ent*-kaurene synthase of gibberellin biosynthesis. - Plant Physiol. **116**: 1271-1278, 1998.

Zeevaart, J.A.D., Gage, D.A.: *Ent*-kaurene biosynthesis is enhanced by long photoperiods in the long-day plants *Spinacia oleracea* L. and *Agrostemma githago* L. - Plant Physiol. **101**: 25-29, 1993.