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Scientific Research

Stress-Induced Flowering in Pharbitis—A Review

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ABSTRACT

Many plant species are induced to flower by stress. Stress-induced flowering has been studied mostly in the short-day plant pharbitis (also called Japanese morning glory; *Ipomoea nil*, formerly *Pharbitis nil*). In this article, physiological characteristics, the regulation by salicylic acid (SA) and the expression of flowering-related genes in stress-induced flowering in pharbitis are reviewed. Pharbitis flowered under long-days in response to poor nutrition or low temperature. The pharbitis plants induced to flower by stress reached anthesis, fruited and produced fertile seeds. The progeny of the stressed plants developed normally. Grafting experiments indicated that a transmissible flowering stimulus is involved in poor nutrition stress-induced flowering. Aminooxyacetic acid (AOA), a phenylalanine ammonia-lyase (PAL) inhibitor, inhibited the stress-induced flowering, and this inhibition was overcome by SA. Stress induced PAL activity and SA biosynthesis. *PnFT2*, a pharbitis ortholog of the flowering gene *FLOWERING LOCUS T* of *Arabidopsis thaliana*, was expressed when the plants were induced to flower by stress. The overexpression of *PnFT2* induced flowering, and *PnFT2*RNAi inhibited it. AOA inhibited *PnFT2* expression induced by stress, and SA eliminated this inhibitory effect. SA enhanced *PnFT2* expression under poor nutrition but not under non-stressful conditions. Therefore, stress may induce the production of SA and other unknown factor(s) that may work in combination to induce *PnFT2* expression and flowering.

Keywords: Flowering; Pharbitis; *PnFT*; Salicylic Acid; Stress

1. Introduction

Flowering in many plant species is regulated by environmental cues such as night length in photoperiodic flowering and temperature in vernalization [1]. In addition, recent studies have indicated that stress is a cue to induce flowering [2-4]. The short-day (SD) plant pharbitis (also called Japanese morning glory; *Ipomoea nil*, formerly *Pharbitis nil*) flowered under long-days (LD) when grown under poor nutrition, low temperature or light conditions of high intensity [5-8]. Poor nutrition, low temperature and high-intensity light are stresses. Accordingly, we named such non-photoperiodic flowering stress-induced flowering [9]. The SD plants *Perilla frutescens* var. *crispa* and *Lemna paucicostata* (also called *Lemna aequinoctialis*) flowered under LD when grown under low-intensity light and poor nutrition, respectively [10,11]. The long-day plant *Arabidopsis thaliana* was induced to flower early under ultraviolet-C (UV-C) radiation and poor nutrition [12,13]. Similar non-photoperiodic flowering has been reported in several plant species, and a review of those reports suggested that most of the factors responsible for flowering are stresses, including high- or low-intensity light, high or low temperature, drought, poor nutrition and mechanical stimulation [2,4]. However, not all types of stress induce flowering in all plants. For example, *P. frutescens* flowers upon low-intensity light stress, but not upon poor nutrition, low temperature, salt or drought stresses [10]. Plants can modify their development to adapt to stressful conditions, and stress-induced flowering is such an adaptation. Plants flower as an emergency response when stressed, thus ensuring their ability to produce the next generation. Through this mechanism, the species is preserved, even under unfavorable environmental conditions. Therefore, stress-induced flowering is universal and as important as photoperiodic flowering and vernalization [2,4]. This article reviews the studies reported on pharbitis because stress-induced flowering has been best studied in this

plant species.

2. Physiological Characteristics of Stress-Induced Flowering in Pharbitis

Pharbitis cv. Violet was induced to flower when grown in 1/10- or 1/100-strength mineral nutrient solution or tap water under LD conditions for 20 days or longer. In contrast, no flowering occurred in the full-strength nutrient solution [14,15]. The vegetative growth of the plants grown in the diluted nutrient solutions was significantly inhibited. Because the suppression of vegetative growth indicated that the plants were stressed [9], the flowering was likely stress-induced. The flowering response was stronger under the stronger stress condition (1/100-strength nutrient solution) than it was under the weaker stress condition (1/10-strength nutrient solution). Another cultivar, Tendan, did not flower under the same poor nutrition conditions, although the vegetative growth was significantly inhibited. Thus, poor nutrient stress does not induce flowering in all cultivars. Cv. Violet was induced to flower when grown at 13˚C or 15˚C for 10 days or longer under LD conditions [9,15]. Vegetative growth was suppressed at low temperatures. Cv. Tendan was also induced to flower by low-temperature stress. The flowering response induced by low temperature was stronger in Tendan than it was in Violet. The flowering response induced by low temperature was weaker than that induced by a single SD treatment in Violet. This trend was reversed in Tendan.

We hypothesized that stressed plants flower to produce the next generation as an emergency response if they cannot adapt to unfavorable environmental conditions. If this is the case, the plants induced to flower by stress must produce fertile seeds, and the progeny must develop normally. Cv. Violet grown in 1/10-strength nutrient solution or tap water throughout its life cycle flowered, reached anthesis, fruited and produced seeds [14]. Those seeds germinated, and the progeny developed normally and responded to SD treatment by forming floral buds. Furthermore, a normal second generation was produced. These data indicate that the stressed plants do not need to await the arrival of a season when photoperiodic conditions are suitable for flowering, and such precocious flowering may assist in species preservation. Therefore, stress-induced flowering may have a biological benefit, and it should be considered as important as photoperiodic flowering and vernalization.

A transmissible flowering stimulus such as florigen, which is involved in photoperiodic flowering, has not been reported in stress-induced flowering. The cotyledons are necessary for the flowering of pharbitis seedlings in response to poor nutrition and low temperature [5,16]. This necessity suggests that a flowering stimulus such as florigen is also involved in stress-induced flowering and that it is produced in cotyledons. If the stress-induced flowering stimulus is transmissible, defoliated scions may flower when grafted onto rootstocks with cotyledons and grown under stressful conditions. Cvs. Violet and Tendan were grafted in several combinations, and the grafted plants were grown in tap water under LD conditions [14]. The Violet scions grafted onto the Violet rootstocks flowered. The flowering may have been due to the rootstocks because all of the leaves were removed from the scions. Therefore, a transmissible flowering stimulus is involved in stress-induced flowering. We predicted that Tendan would not produce such a flowering stimulus because this cultivar did not flower under poor nutrition conditions. If this were the case, Violet would not be expected to flower when grafted onto Tendan rootstocks. However, defoliated Violet scions grafted onto Tendan rootstocks with cotyledons flowered. Conversely, Tendan scions did not flower when grafted onto Violet rootstocks. These results indicate that Tendan produces a transmissible flowering stimulus but does not respond to it.

3. Regulation of Stress-Induced Flowering by Salicylic Acid (SA)

When pharbitis was induced to flower by poor nutrition, the plants turned red due to the accumulation of anthocyanins, whose biosynthesis is regulated by phenylalanine ammonia-lyase (PAL). The activity of PAL increases when plants are stressed [17,18]. These results suggest that the metabolic pathway mediated by PAL is involved in the regulation of stress-induced flowering. Aminooxyacetic acid (AOA) and L-2-aminooxy-3-phenylpropionic acid, which are PAL inhibitors [19,20], inhibited stress-induced flowering in pharbitis [9,14]. PAL catalyzes the conversion of phenylalanine to *t*-cinnamic acid, from which anthocyanins, chlorogenic acid and SA are produced. We inhibited stress-induced flowering by applying AOA in combination with various metabolites. Among them, *t*-cinnamic acid, benzoic acid (a precursor of SA) and SA negated the inhibitory effect of AOA, while *p*-coumaric and caffeic acids, which lead to the synthesis of anthocyanins and chlorogenic acid, did not [9,14]. Flowering was induced by growing the plants in a 1/100-strength nutrient solution, and *PAL* gene expression in the cotyledons harvested at the end of the stress treatment was examined by reverse transcription-polymerase chain reaction (RT-PCR). The expression was stronger in the plants that were induced to flower by stress than in the control plants [21]. The plants were then induced to flower in the same manner as above, and PAL enzyme activity in the cotyledons was measured. The PAL activity was higher in the stressed plants than in the control plants. The SA content in the cotyledons was also measured by high performance liq-

uid chromatography-mass spectrometry. It was higher in the stressed plants than in the control plants. These results suggest that poor nutrition stress-induced flowering in pharbitis is induced by SA synthesis, which is promoted by PAL.

The effect of exogenously applied SA on flowering was examined. SA did not induce flowering under non-stressful conditions, but it promoted flowering under stressful conditions (unpublished data). Therefore, SA may be necessary but not sufficient to induce flowering. Stress may induce the production of not only SA but also other essential factors involved in the induction of flowering.

It has long been supposed that SA is involved in the regulation of photoperiodic flowering in the Lemnaceous plants because SA induces flowering in *L. paucicostata*, *Lemna gibba* and some other species belonging to the Lemnaceae [22-24]. However, no evidence has shown an increase in the SA content under flowering conditions. When *L. paucicostata* was induced to flower by poor nutrition, the SA content in the fronds increased [11]. This is the first evidence that the SA content increases when flowering is induced in a Lemnaceous species. SA may function as an endogenous regulating factor in stress-induced flowering but not in photoperiodic flowering. The involvement of SA was also reported in the stress-induced flowering of *A. thaliana* [12,25]. These data support the conclusions for pharbitis described above. The regulatory mechanism of stress-induced flowering mediated by SA may be common among various plant species.

4. Genetic Regulation of Stress-Induced Flowering

The molecular basis of the regulation of stress-induced flowering is not well understood. *A. thaliana* flowering is induced by LD, vernalization, autonomous cues and gibberellins, and these factors operate through a common pathway integrated by the floral pathway integrator gene *FLOWERING LOCUS T* (*FT*) [26]. This suggests that *FT* could also be involved in stress-induced flowering. Two orthologs of *FT*, *PnFT1* and *PnFT2*, have been identified in pharbitis, and these genes are expressed under inductive SD conditions to promote flowering [27]. Therefore, we examined *PnFT* expression in stress-induced flowering by RT-PCR. Pharbitis cv. Violet was grown in a 1/100-strength nutrient solution under LD conditions, and a control group of plants was given a single SD treatment without the stress treatment as a positive control. *PnFT1* and *PnFT2* were induced in the cotyledons when flowering was induced by the SD treatment, but neither gene was expressed without the SD or the stress treatment [14,15]. *PnFT2* was induced in the plants that flowered under the poor nutrition conditions for two weeks or longer. The level of mRNA expression closely correlated with the flowering response. Conversely, *PnFT1* was not expressed when flowering was induced by poor nutrition. *PnFT2* expression in cv. Tendan, in which poor nutrition does not induce flowering, was also examined. The expression level was similar in plants grown under both poor nutrition and non-stressful conditions and was much lower than in the SD-treated cotyledons. *PnFT2* expression in the stressed leaves was weaker in Tendan than in Violet. *PnFT1* expression was also not induced by poor nutrition in Tendan. These results suggest that *PnFT2*, but not *PnFT1*, is the major regulatory gene involved in the stress-induced flowering of pharbitis. Violet was induced to flower by growing it at 15˚C for 2 weeks or longer, and the expression of *PnFT2* was detected starting at the first week of the low temperature treatment [15]. *PnFT1* expression was also detected, but a significant increase was not observed until the fourth week of the stress treatment. The low temperature induced *PnFT2* expression, and *PnFT1* expression was quite weak in Tendan as well as in Violet. *PnFT2* expression was stronger in Tendan than in Violet. These results indicate that *PnFT2* is also involved in low temperature stress-induced flowering. The involvement of *PnFT2* in stress-induced flowering in pharbitis is consistent with a previous report indicating that *FT* is involved in UV-C stress-induced flowering in *A. thaliana* [12]. It is interesting to note that *PnFT2* is involved in both photoperiodic flowering and stress-induced flowering, whereas *PnFT1* is involved only in photoperiodic flowering in pharbitis. The two PnFT proteins might have different roles in the regulation of flowering depending on the inductive cue. *PnFT2* and *PnFT2*RNAi were transformed into *A. thaliana* and pharbitis to test whether *PnFT2* has flower-inducing activity (Yamada, unpublished data). *A. thaliana* transformed with *35S::PnFT2* flowered much earlier than the wild type under continuous light. Some pharbitis somatic embryoids transformed with *35S::PnFT2* flowered without regeneration of vegetative shoots during cultivation on regeneration medium. Stress-induced and SD-induced flowering were suppressed in the pharbitis plants transformed with *PnFT2*RNAi. These results indicate that *PnFT2* has flower-inducing activity.

SUPPRESSOR OF OVEREXPRESSION OF CO1 (*SOC1*) is thought to be another floral pathway integrator gene in *A. thaliana* [28,29]. Therefore, it was expected that *SOC1* expression might also be induced when flowering was induced by stress in pharbitis. However, the pharbitis *SOC1* homolog was expressed constitutively and showed no correlation with flowering response [15]. It was reported that UV-C, which induced flowering, did

not induce *SOC1* expression in *A. thaliana* [12]. These results suggest that *SOC1* is not involved in stress-induced flowering. *CONSTANS* (*CO*), a gene upstream of *FT*, is involved in the regulation of photoperiodic flowering in many plants [30]. The CO protein directly induces the transcription of *FT* in *A. thaliana* and *Oryza sativa* [31-33]. It has been proposed that the CO/FT regulatory module (*i.e.*, the CO protein activates *FT* transcription) is highly conserved in both dicot and monocot plants [34]. The expression of *CO* was also moderately induced in UV-C-induced flowering in *A. thaliana* [12]. The pharbitis homolog of *CO*, *PnCO*, complements the *co* mutation in *A. thaliana* [35]. We found, however, that *PnCO* is constitutively expressed regardless of the flowering status in pharbitis; it is expressed under both rich and poor nutrition and both SD and LD conditions [15]. These results suggest that *PnCO* may not be involved in the regulation of both stress-induced and photoperiodic flowering in pharbitis. This conclusion is consistent with the previous report that *PnFT* mRNA abundance was not related to *PnCO* expression and that therefore *PnFT* may not be regulated by the PnCO protein in pharbitis [27,36]. Furthermore, a night-break treatment inhibited flowering but did not influence *PnCO* expression [35]. The CO/FT module may not be conserved in pharbitis.

RT-PCR on the genes that act upstream of *FT* showed that the expression of the homologs of *FLOWERING LOCUS C*, *FRIGIDA* and *FVE* was not induced by poor nutrition, and the *FCA* homolog was expressed even in the absence of stress [15]. Thus, candidate genes that act upstream of and regulate *FT* expression were not identified among the known flowering genes. Among the genes downstream of *FT*, the expression of the *APETALA1* homolog correlated well with the flowering response, but the expression of the *FD* homolog was not detected (Yamada, unpublished data).

5. Interaction between SA and *PnFT2* in the Regulation of Stress-Induced Flowering

We hypothesized that stress-induced flowering is regulated by *PnFT*, which is induced by SA. Accordingly, we examined the influences of both a PAL inhibitor and SA on *PnFT* expression in pharbitis. AOA inhibited flowering and *PnFT2* expression induced by poor nutrition, and SA eliminated the inhibitory effects of AOA [15]. SA enhanced *PnFT2* expression under poor nutrition but not under non-stressful conditions. These results suggest that SA induces *PnFT2* expression, which in turn induces flowering. This conclusion is consistent with the results observed in *A. thaliana* and sunflower, in which SA induced the expression of *FT* and *HAFT*, a sunflower ortholog of *FT* [12,37]. However, SA did not induce *PnFT2* expression and flowering under non-stressful conditions in pharbitis, suggesting that SA alone may not be sufficient to induce *PnFT2* expression. Stress may induce the production of SA and other unknown factor(s), which may work in combination to induce *PnFT2* expression and flowering.

6. Conclusion

Pharbitis is typically induced to flower under SD conditions and can be induced to flower under unfavorable photoperiodic conditions when exposed to stress. Stress activates PAL and enhances SA levels, promoting *PnFT2* expression to induce flowering. However, SA alone cannot induce flowering, and other factors are necessary. The regulation of stress-induced flowering by SA may be common, at least in *L. paucicostata* and *A. thaliana*.

REFERENCES

[1] B. Thomas and D. Vince-Prue, "Photoperiodism in Plants," Academic Press, San Diego, 1997.

[2] K. C. Wada and K. Takeno, "Stress-Induced Flowering," *Plant Signaling and Behavior*, Vol. 5, No. 8, 2010, pp. 1-4.

[3] K. C. Wada and K. Takeno, "Salicylic Acid-Mediated Stress-Induced Flowering," In: S. Hayat, A. Ahmad and M. N. Alyemini, Eds., *Salicylic Acid*: *Plant Growth and Development*, Springer, New York, 2013, pp. 163-182. http://dx.doi.org/10.1007/978-94-007-6428-6_9

[4] K. Takeno, "Stress-Induced Flowering," In: P. Ahmad and M. N. V. Prasad, Eds., *Abiotic Stress Responses in Plants*: *Metabolism*, *Productivity and Sustainabil*ity, Springer, New York, 2012, pp. 331-345. http://dx.doi.org/10.1007/978-1-4614-0634-1_17

[5] M. Shinozaki and A. Takimoto, "Effects of Some Growth Regulators and Benzoic Acid Derivatives on Flower Initiation and Root Elongation of *Pharbitis nil*, Strain Kidachi," *Plant and Cell Physiology*, Vol. 24, No. 3, 1983, pp. 433-439.

[6] M. Shinozaki, K. Asada and A. Takimoto, "Correlation between Chlorogenic Acid Content in Cotyledons and Flowering in *Pharbitis* Seedlings under Poor Nutrition," *Plant and Cell Physiology*, Vol. 29, No. 4, 1988, pp. 605-609.

[7] M. Shinozaki, N. Hirai, Y. Kojima, K. Koshimizu and A.Takimoto, "Correlation between Level of Phenylpropanoids in Cotyledons and Flowering in *Pharbitis* Seedlings under High-fluence Illumination," *Plant and Cell Physiology*, Vol. 35, No. 5, 1994, pp. 807-810.

[8] A. Ishimaru, K. Takeno and M. Shinozaki, "Correlation of Flowering Induced by Low Temperature and Endogenous Levels of Phenylpropanoids in *Pharbitis nil*: A Study with a Secondary-Metabolism Mutant," *Journal of Plant Physiology*, Vol. 148, No. 6, 1996, pp. 672-676. http://dx.doi.org/10.1016/S0176-1617(96)80366-X

[9] T. Hatayama and K. Takeno, "The Metabolic Pathway of Salicylic Acid Rather than of Chlorogenic Acid Is Involved in the Stress-Induced Flowering of *Pharbitis nil*,"

Journal of Plant Physiology, Vol. 160, No. 5, 2003, pp. 461-467. http://dx.doi.org/10.1078/0176-1617-01041

[10] K. C. Wada, H. Kondo and K. Takeno, "Obligatory Short-Day Plant, *Perilla frutescens* var. *crispa* Can Flower in Response to Low-intensity Light Stress under Long-Day Conditions," *Physiologia Plantarum*, Vol. 138, No. 3, 2010, pp. 339-345. http://dx.doi.org/10.1111/j.1399-3054.2009.01337.x

[11] A. Shimakawa, T. Shiraya, Y. Ishizuka, K. C. Wada, T. Mitsui and K. Takeno, "Salicylic Acid Is Involved in the Regulation of Starvation Stress-Induced Flowering in *Lemna paucicostata*," *Journal of Plant Physiology*, Vol. 169, No. 10, 2012, pp. 987-991. http://dx.doi.org/10.1016/j.jplph.2012.02.009

[12] C. Martínez, E. Pons, G. Prats and J. León, "Salicylic Acid Regulates Flowering Time and Links Defense Responses and Reproductive Development," *Plant Journal*, Vol. 37, No. 2, 2004, pp. 209-217. http://dx.doi.org/10.1046/j.1365-313X.2003.01954.x

[13] J. Kolář and J. Seňková, "Reduction of Mineral Nutrient Availability Accelerates Flowering of *Arabidopsis thaliana*," *Journal of Plant Physiology*, Vol. 165, No. 15, 2008, pp. 1601-1609. http://dx.doi.org/10.1016/j.jplph.2007.11.010

[14] K. C. Wada, M. Yamada, T. Shiraya and K. Takeno, "Salicylic Acid and the Flowering Gene *FLOWERING LOCUS T* Homolog Are Involved in Poor-Nutrition Stress-Induced Flowering of *Pharbitis nil*," *Journal of Plant Physiology*, Vol. 167, No. 6, 2010, pp. 447-452. http://dx.doi.org/10.1016/j.jplph.2009.10.006

[15] M. Yamada and K. Takeno, "Stress and Salicylic Acid Induce the Expression of *PnFT2* in the Regulation of the Stress-Induced Flowering of *Pharbitis nil*," *Journal of Plant Physiology*, 2013. http://dx.doi.org/10.1016/j.jplph.2013.07.005

[16] M. Shinozaki, "Organ Correlation in Long-Day Flowering of *Pharbitis nil*," *Biologia Plantarum*, Vol. 27, No. 4-5, 1985, pp. 382-385. http://dx.doi.org/10.1007/BF02879882

[17] R. A. Dixon and L. Paiva, "Stress-Induced Phenylpropanoid Metabolism," *Plant Cell*, Vol. 7, No. 7, 1995, pp. 1085-1097.

[18] I. M. Scott, S. M. Clarke, J. E. Wood and L. A. J. Mur, "Salicylate Accumulation Inhibits Growth at Chilling Temperature in *Arabidopsis*," *Plant Physiology*, Vol. 135, No. 2, 2004, pp. 1040-1049. http://dx.doi.org/10.1104/pp.104.041293

[19] H. Kessmann, R. Edwards, P. W, Geno and R. A. Dixon, "Stress Responses in Alfalfa (*Medicago sativa* L.): V. Constitutive and Elicitor-induced Accumulation of Isoflavonoid Conjugates in Cell Suspension Cultures," *Plant Physiology*, Vol. 94, No. 1, 1990, pp. 227-232. http://dx.doi.org/10.1104/pp.94.1.227

[20] C. Appert, J. Zoń and N. Amrhein, "Kinetic Analysis of the Inhibition of Phenylalanine Ammonia-Lyase by 2-Aminoindan-2-phosphonic Acid and Other Phenylalanine Analogues," *Phytochemistry*, Vol. 62, No. 3, 2003, pp. 415-422. http://dx.doi.org/10.1016/S0031-9422(02)00561-7

[21] K. C. Wada, "Regulatory Mechanism of Stress-Induced Flowering," Ph.D. Dissertation, Niigata University, Niigata, 2012.

[22] C. F. Cleland and A. Ajami, "Identification of the Flower-Inducing Factor Isolated from Aphid Honeydew as Being Salicylic Acid," *Plant Physiology*, Vol. 54, No. 6, 1974, pp. 904-906. http://dx.doi.org/10.1104/pp.54.6.904

[23] C. F. Cleland, O. Tanaka and L. J. Feldman, "Influence of Plant Growth Substances and Salicylic Acid on Flowering and Growth in the Lemnaceae (Duckweeds)," *Aquatic Botany*, Vol. 13, 1982, pp. 3-20. http://dx.doi.org/10.1016/0304-3770(82)90036-5

[24] R. Kandeler, "Lemnaceae," In: A. H. Halevy, Ed., *CRC Handbook of Flowering*, Vol. 3, CRC Press, Boca Raton, 1985, pp. 251-279.

[25] L. Corbesier and G. Coupland, "Photoperiodic Flowering of *Arabidopsis*: Integrating Genetic and Physiological Approaches to Characterization of the Floral Stimulus," *Plant and Cell Environment*, Vol. 28, No. 1, 2005, pp. 54-66. http://dx.doi.org/10.1111/j.1365-3040.2005.01283.x

[26] P. K. Boss, R. M. Bastow, J. S. Mylne and C. Dean, "Multiple Pathways in the Decision to Flower: Enabling, Promoting, and Resetting," *Plant Cell*, Vol. 16, No. S1, 2004, pp. S18-S31. http://dx.doi.org/10.1105/tpc.015958

[27] R. Hayama, B. Agashea, E. Luleya, R. King and G. Coupland, "A Circadian Rhythm Set by Dusk Determines the Expression of *FT* Homologs and the Short-Day Photoperiodic Flowering Response in Pharbitis," *Plant Cell*, Vol. 19, No. 10, 2007, pp. 2988-3000. http://dx.doi.org/10.1105/tpc.107.052480

[28] J. Lee and I. Lee, "Regulation and Function of *SOC1*, a Flowering Pathway Integrator," *Journal of Experimental Botany*, Vol. 61, No. 9, 2010, pp. 2247-2254. http://dx.doi.org/10.1093/jxb/erq098

[29] R. Amasino, "Seasonal and Developmental Timing of Flowering," *Plant Journal*, Vol. 61, No. 6, 2010, pp. 1001-1013. http://dx.doi.org/10.1111/j.1365-313X.2010.04148.x

[30] F. Valverde, "CONSTANS and the Evolutionary Origin of Photoperiodic Timing of Flowering," *Journal of Experimental Botany*, Vol. 62, No. 8, 2011, pp. 2453-2463. http://dx.doi.org/10.1093/jxb/erq449

[31] P. Suárez-López, K. Wheatley, F. Robson, H. Onouchi, F. Valverde and G. Coupland, "*CONSTANS* Mediates the Circadian Clock and the Control of Flowering in *Arabidopsis*," *Nature*, Vol. 410, No. 6832, 2001, pp. 1116-1120. http://dx.doi.org/10.1038/35074138

[32] T. Izawa, T. Oikawa, N. Sugiyama, T. Tanisaka, M. Yano and K. Shimamoto, "Phytochrome Mediates the External Light Signal to Repress *FT* Orthologs in Photoperiodic Flowering of Rice," *Genes and Development*, Vol. 16, No. 15, 2002, pp. 2006-2020. http://dx.doi.org/10.1101/gad.999202

[33] F. Valverde, A. Mouradov, W. Soppe, D. Ravenscroft, A. Samach and G. Coupland, "Photoreceptor Regulation of CONSTANS Protein in Photoperiodic Flowering," *Science*, Vol. 303, No. 5660, 2004, pp. 1003-1006.

http://dx.doi.org/10.1126/science.1091761

[34] I. G. Matsoukas, A. J. Massiah and B. Thomas, "Florigenic and Antiflorigenic Signaling in Plants," *Plant and Cell Physiology*, Vol. 53, No. 11, 2012, pp. 1827-1842. http://dx.doi.org/10.1093/pcp/pcs130

[35] J. Liu, J. Yu, L. McIntosh, H. Kende and J. A. D. Zeevaart, "Isolation of a *CONSTANS* Ortholog from *Pharbitis nil* and its Role in Flowering," *Plant Physiology*, Vol. 125, No. 4, 2001, pp. 1821-1830. http://dx.doi.org/10.1104/pp.125.4.1821

[36] Y. Higuchi, K. Sage-Ono, R. Sasaki, N. Ohtsuki, A. Hoshino, S. Iida, H. Kamada and M. Ono, "Constitutive Expression of the *GIGANTEA* Ortholog Affects Circadian Rhythms and Suppresses One-Shot Induction of Flowering in *Pharbitis nil*, a Typical Short-Day Plant," *Plant and Cell Physiology*, Vol. 52, No. 4, 2011, pp. 638-650. http://dx.doi.org/10.1093/pcp/pcr023

[37] C. A. Dezar, J. I. Giacomelli, P. A. Manavella, D. A. Ré, M. Alves-Ferreira, I. T. Baldwin, G. Bonaventure and R. L. Chan, "HAHB10, a Sunflower HD-Zip II Transcription Factor, Participates in the Induction of Flowering and in the Control of Phytohormone-mediated Responses to Biotic Stress," *Journal of Experimental Botany*, Vol. 62, No. 3, 2011, pp. 1061-1076. http://dx.doi.org/10.1093/jxb/erq339