博士論文

Studies on the regulation of diurnal flower opening and closure rhythms by circadian clocks

（概日時計による花の日周期開閉運動の
制御に関する研究）

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Abstract

Many ornamentals show flower opening and closing oscillation of diurnal rhythm (Shimizu-Yumoto and Ichimura, 2012). Flower opening in the daytime and closure during night has some advantages for plants, since many pollinators are active during the day, promising successful pollination, fertilization and fruit set. However, as ornamentals, which are displayed at flower shops and appreciated by consumers not only during the day but also at night, the ability to keep fully open status of flowers at night or in a specific time of the day is an important trait. Therefore, development of techniques to control the rhythms or breeding of cultivars that can keep open flowers at any time of the day as consumers prefer would have potential demands.

Eustoma grandiflorum (Raf.) Shinn, belonging to *Eustoma ressellianum*, Gentianaceae is native to a warm regions of the Southern United States, Mexico, Caribbean and northern South America where the minimum temperature is about 10 °C in winter and the maximum temperature is 30 °C or higher in summer (Ohkawa et al., 1991; Shinners, 1957). *E. grandiflorum* was introduced into Japan more than 60 years ago (Ohkawa et al., 1991). Since then, many cultivars having a wide diversity of colors, shapes, and sizes have been released (Harbaugh, 2006; Hisamatsu et al., 1998). Thus, it has become a promising flower in the cut flower market in Japan and in the world. To date, there are so many studies on *E. grandiflorum* from the flower postharvest quality, senescence to mechanism of flower shape (Kawabata et al., 2011; Shimizu-Yumoto and Ichimura, 2010; Shimizu-Yumoto and Ichimura, 2012). *E. grandiflorum* flowers open in the morning and close at dusk for several consecutive days. These rhythmic movements in petals of *E. grandiflorum* have not been studied. The purpose of this study was to investigate flower opening and closure rhythms synchronized with environmental light rhythms for

establishing the basis for controlling and extending the period of fully open status of the flowers. In this study, we performed various light conditions to investigate the effect of light on *E. grandiflorum*.

Tomato as an important model plant, own many mutants in photomorphogenesis (Kendrick et al., 1997). Many phytochrome and cryptochrome mutants obtained in tomato, such as mutant *phyA*, *phyB* and *cry1* (Giliberto et al., 2005; Kendrick et al., 1997; Van Tuinen et al., 1995). The flowers of tomato also show reciprocal oscillations of flower opening and closure. We use different photoreceptor-defect mutants to verify the effect of the different light on diurnal flower opening and closure.

The results showed that, flower opening and closing rhythm was directly regulated by light and circadian in *E. grandiflorum*. This diurnal movement was exactly synchronized to environmental light/dark cycles. We also found that minimum requirement of dark period would be around 4 hours in *E. grandiflorum* flowers. Our data showed that red light or blue light was sufficient for the synchronization of flower opening and closure rhythms, indicating that flower opening and closing rhythm is controlled by multiple photoreceptors. Both cryptochromes and phytochromes seem to be involved, but the involvement of other photoreceptors for blue light would be possible.

To clarify the regulation of each photoreceptor on flower opening and closing rhythm, we performed the treatments for conversion of red/far-red light as well as light-break. The data showed that red light plays a dominant role in maintaining the amplitudes of oscillations, but also overcomes the inhibition of flowering which is produced by far-red light.

We make use of model plant tomato to investigate the role of cryptochromes and phytochromes in regulation of circadian and flower opening and closure. Under continuous light conditions (LL), circadian rhythm persisted in the phyB deficient mutant. Comparing of mutant *phyB2-1*, mutant *phyB1-1* showed obvious oscillation rhythm when under

LL condition. In addition, dual mutant of *phyB* deficiency and triple mutant *phyAphyB1-1phyB2-1* exhibited normal oscillations, while rhythm was disappeared in other mutants where *phyB* gene was activated. Under continuous dark conditions, all *cry1*-deficien- mutants showed a shortened length of circadian period. Take together, it was suggested that red light played a role in mediating the amplitude of oscillations and blue light was related with length of oscillation in circadian clock.

Chapter 1 Introduction

1.1 Flower opening and closure

So far, flower diurnal opening and closure have been reported in many varieties of cut flower (Shimizu-Yumoto and Ichimura, 2012), such as rose (Horibe and Yamada, 2014) and *E. grandiflorum* (exhibited in this work). Flower opening and closing, in most of species studied, are due to: 1) Different growth rate in two sides of petal. The opening of some species mainly responds to temperature. For example, in crocus and tulip, high temperature could promote growth rapidly in inner surface of petal (Pfeffer, 1873; van Doorn and van Meeteren, 2003; Wiedersheim, 1904). 2) Movement of midrib. Different cell expansion in midrib will cause the movement in petals (Kaihara and Takimoto, 1981; Phillips Jr and Kende, 1980). 3) Different water content in cells of petal during day and night. For example, in *Silene saxifrage* flowers, loss of water resulted in petal closing, and when the cells refill with water, the petal will open (Halket, 1931). 4) Reversible expansion and contraction of cells. In *Gentiana kochiana*, the change of cell's size due to turgor changes in both inner and outer epidermis, resulted in rhythm movement of petal (van Doorn and van Meeteren, 2003). In the following sections, we will elaborate the mechanism of flower movement from the physiology aspect of the growth.

Carbohydrate metabolism

In some plants, the mobilization of storage carbohydrates will be different between flower closure and opening. The cells of young petal in many species contain a large amount of starch. These starch can be rapidly converted to glucose and fructose when flowers are closed to opening (Hammond, 1982; Ho and Nichols, 1977). For example, flowers of *Tradescanti*a reflexa (Horie, 1961), *Alstroemeria peregrina* (Collier, 1997), Turnera ulmifolia (Ball, 1933), *Lilium* (Bieleski et al., 2000a), and *Magnolia grandiflora* (Griesel, 1954), contain a high concentration of starch. By contrast, petals of daylily flowers (*Hemerocallis* sp.), contain a high concentration of fructan not starch when it closed. Fructan is rapidly

degraded when flower are closed to opening (Bieleski, 1993). In some circumstance, flower opening is due to a coordination of uptake and degradation of various polysaccharides. In freesia florets, for example, the increase in perianth sugars was more than 10 times higher than the decrease in starch content. It indicated that sugar was absorbed (Van Meeteren et al., 1995). The similar phenomenon that the increase in sugar content was higher than the decrease in starch content, was showed in gladiolus florets (Yamane et al., 1991).

Cell wall expansion

So far, the changes in the cell wall of flowers growth and opening doesn't attract people's enough attention (O'Donoghue et al., 2002a; O'Donoghue et al., 2002b). Recently, it was proposed that, the extensibility of cell wall is a limiting factor in petal growth and flower opening. Yamada and Ochiai found that flower opening is accompanied by an increment of cell wall extensibility in rose and *E. grandiflorum* (Kunio Yamada et al., 2009; Ochiai et al., 2013).

Hormonal regulation

Except for ethylene, the role of regulation by endogenous hormones is as yet unclear. Ethylene can promote or inhibit flower opening in different species even different cultivars (Reid et al., 1989). The endogenous ethylene play a negative role in flowers opening in potted rose plants (Cushman et al., 1994; Tjosvold et al., 1994), such as cut rose flowers (Yamamoto et al., 1994) and cut gladiolus inflorescences (Serek et al., 1994). It has been reported that ABA (Abscisic Acid) can promote flowers opening in *Ipomoea nil*. However, the effect of ABA in *Ipomoea nil* may be due to the increase of ethylene production (Koning, 1986) and this effect could be eliminated by amino ethoxyvinyl glycine and cobalt ions which are the ethylene biosynthesis inhibitors. IAA (Indole-3-acetic Acid), another plant hormone which as a inhibitor, shows a negative function in *Ipomoea* flower opening (Kaihara and Takimoto, 1983). According to the

different organizations and concentrations, IAA can promote or inhibit ethylene production and the effect of IAA in *Ipomoea* may also be regulated by ethylene.

In *Arabidopsis*, a mutant *defective in anther dehiscence1 (dad1)* affecting flower opening was identified. The defect could be rescued by an exogenous JA (jasmonic acid) or linolenic acid. The DAD 1 gene was shown to be related with jasmonic acid synthesis. Thus, JA may also be involved in flower opening in some species (Ishiguro et al., 2001).

1.2 Circadian rhythm in organisms

The biological process of most living organisms that includes metabolism, physiology and behavior displays an endogenous, entrainable oscillation of approximately 24 hours (Dunlap et al., 2004). This periodicity (called circadian rhythm) as an adaptation to the cycle of day and night is generated by the rotation of the earth. These rhythms have been described extensively in mammals, insects, fungus and plants, demonstrate as physiological changes, such as sleeping and feeding in animas, leaf rhythmic movement, photoperiodic flowering and hypocotyls elongation in plant (Dunlap, 1999; McClung, 2006; Niinuma et al., 2007).

Generally, circadian rhythms are defined by these three general parameters (Dunlap et al., 2004): 1) The rhythm has an endogenous free-running period that sustains about 24 hours. It can be persisted in continuous environmental conditions, continuous light (or dark), and continuous temperature. That is, under these extreme conditions, the free-running period of approximately 24 hours could be observed in the organisms; 2) The rhythms are entrainable. These rhythms can be reset by exposure to external environmental stimulus (such as light and high temperature). Under the controlled circumstances (such as in the laboratory), environmental time cues (light/dark cycles and/or temperature cycles) derive from the alternation of day and night. These external stimulus used to entrain a rhythm with a period of 24 hours is called the Zeitgeber, or "Time giver" (McClung, 2006). 3) All circadian rhythms exhibit temperature compensation. The period maintains relatively constant circadian periodicity over a range of physiological temperatures (Pittendrigh, 1954).

Diurnal rhythm was first recognized as early as fourth century BC, however, the scientific literature on circadian began in 1729. French astronomer de Mairan reported that, the sensitive heliotrope plant (probably *Mimosa pudica*) show the diurnal leaf movements in constant darkness

condition, demonstrating their endogenous origin (de Mairan, 1729). The endogenous property not simply responses to environmental time cues of leaf movement rhythms was demonstrated in the 18th century (de Mairan, 1729; Duhamel DuMonceau, 1759). Now, at 21st century, the molecular details of plant circadian systems were extensively being investigated (Table. 1-1) (Love et al., 2004; Yakir et al., 2007).

Table 1-1. The circadian system has a regulatory role in nearly all aspects of a plant's life.

Rhythmical patterns	**Examples**	**References**
Germination	*Betula pubescens* *Diapensia lapponica* *Chamaedaphne calyculata*	(Baskin and Baskin, 1976; Black and Wareing, 1954; Densmore, 1997)
Hypocotyl elongation	*Solanum esculentumm* *Arabidopsis thaliana* *Chenopodium rubrum*	(Dowson - Day and Millar, 1999; Fernandez and Wagner, 1994; Lecharny and Wagner, 1984; Tukey Jr and Ketellapper, 1963)
Leaf movements	Legumes *Arabidopsis thaliana*	(Dowson - Day and Millar, 1999; Webb, 2003)
Circumnutations	*Arabidopsis thaliana*	(Niinuma et al., 2005)
Shade avoidance	*Arabidopsis thaliana*	(Salter et al., 2003)
Flowering time	*Arabidopsis thaliana*	(Huang et al., 2005; Searle and Coupland, 2004)
Flower opening	*Cestrum nocturnum* *Arabidopsis thaliana*	(Overland, 1960; van Doorn and van Meeteren, 2003)

Table 1-1 (continued)

Rhythmical patterns	**Examples**	**References**
Tuberization	*Solanum tuberosum*	(Martínez-García et al., 2002)
Winter dormancy	*Castanea sativa* *Populus tremula*	(Böhlenius et al., 2006; Ramos et al., 2005)
Stomatal opening	*Arabidopsis thaliana*	(Gorton et al., 1993; Somers et al., 1998b)
Photosynthesis	*Arabidopsis thaliana*	(Harmer et al., 2000; Lu et al., 2005; Schaffer et al., 2001)
Photoprotection	*Arabidopsis thaliana*	(Harmer et al., 2000)
Protection from temperature extremes	*Arabidopsis thaliana*	(Harmer et al., 2000)
Scent production	*Nicotiana suaveolens* *Antirrhinum majus*	(Kolosova et al., 2001; Pesti, 1976)

1.2.1 Circadian rhythms in plants

Like many other organisms, plant also exhibit many rhythmical process in physiological, metabolic and developmental which are driven by endogenous biological clocks (Love et al., 2004; Yakir et al., 2007).

Generally, a circadian system can be divided into three conceptual parts: input pathways that entrain the clock, the central oscillator, and output pathways that generate various rhythmical oscillations (McClung, 2001). Plant clocks are synchronized by environmental cures changer in light or temperature (Mas and Yanovsky, 2009). The external synchronizing cues facilitate to adjust the endogenous clock period to precisely suit the environmental cycle. Entraining stimuli include light, (mediated through phytochromes (PHY) and cryptochromes (CRY) as well as interaction among them and their downstream signalling pathways (Casal and Yanovsky, 2005; Somers et al., 1998a; Yanovsky and Kay, 2001), temperature (Bünning and Moser, 1973; Liu et al., 1998), and imbibitions (Tischkau et al., 2000). Detail of light cues will be described in 1.3 of chapter 1.

Rhythm output

1) *Movement and growth rhythm*

Many life organs displayed rhythmic phenomenon, which included the classic system of pulvinar leaf movement. Pulvinar as a motor organ is responsible for the movement of leaves and leaflets, regulated by diurnal and circadian rhythms. The movements caused by coordinated and simultaneous volume changes of cells on opposing sides of the pulvinus. This rhythmic movement is driven by swelling of extensor and flexor regions of pulvinus (Engelmann and Johnsson, 1998). Ion fluxes result in volume changes, then cells is driven to swell and shrink (Kim et al., 1993). More recently, it was demonstrated that the rhythmic expression of Potassium channels genes Spick1, Spick2, Spork1, and Spock1 in the leguminous Mimosacea tree *Samanea saman* was regulated by light and the

circadian clock. Based on northern blot analysis, their transcript level is correlated with the rhythmic leaf movements (Moshelion et al., 2002).

There are also circadian rhythms affecting growth rate and cell elongation of plant. For example, hypocotyl growth of *Arabidopsis* exhibiting a circadian oscillation in elongation rate is regulated by light and hormones (Halliday and Fankhauser, 2003; Nemhauser and Chory, 2002; Symons and Reid, 2003). In constant light, the patterns of hypocotyl elongation is inhibited at subjective dawn and have an interval of rapid growth at subjective dusk. The rhythm of was entrained by light–dark cycles. This rhythm period of hypocotyl elongation was shortened in the *toc1–1* mutant, indicating that it is controlled by a similar circadian system to other rhythmic markers (Dowson‐Day and Millar, 1999).

2) *Stomatal Aperture, Gas Exchange and CO_2 Assimilation*

Stomata, the small pores exist on the aerial surfaces of leaves and stalks in most plants, bounded by a pair of guard cells that allow gas exchange between the plant and the atmosphere. Circadian rhythms in stomatal aperture have been described in a number of systems (Lumsden and Millar, 1998; Satter et al., 1990). Stomata contribute to control CO_2 uptake and transpirational water loss, and they are modulated by light, relative humidity (Kircher et al.), wind speed and CO_2 concentration (Schulze et al., 1987; Sharkey et al., 1987). In addition to environmental factors, stomata also are regulated by an endogenous circadian clock, although under constant conditions (Gorton et al., 1993; Gorton et al., 1989; Ritz and Kluge, 1987). Circadian control caused that stomata opened during the day and closed at night in most plants. In beans, Calvin cycle reactions, stomatal aperture and gas exchange are regulated by circadian clock (Hennessey and Field, 1991).

3) *Rhythms in gene expression*

Variety of genes are regulated by circadian clock with rhythmic expression (Robertson McClung, 2000; Somers, 1999). In plant, the

circadian which regulates rhythmic gene expression, is present at each step: transcription (Liu et al., 1996; Millar and Kay, 1991; Millar and Kay, 1996), transcript abundance(Fujiwara et al., 1996; Zheng et al., 1998), translation (Staiger et al., 2003), and posttranslational processing (Niramo, 1998). In *Arabidopsis*, there are approximately 5%-6% genes are rhythmically expressed (Harmer et al., 2000). For instance, mRNA abundance of the CAT3 and CAT2 catalase genes peaks at dusk and dawn, respectively (Zhong and McClung, 1996). The temporal gene expression in central oscillator results in that the output pathway from the oscillator to gene expression is very short compared with some complex processes like cell expansion and flowering.

4) *Calcium*

As a second message in plant signalling pathway (Sanders et al., 1999), Calcium plays a key role in guard cell signalling (Leckie et al., 1998; Schroeder et al., 2001) and is involved in circadian regulation of gas exchange and stomatal aperture. Free Ca^{2+} oscillates with a circadian rhythm in the cytosol and chloroplast of *N. plumbaginifolia* and in the cytosol of *Arabidopsis* (Johnson et al., 1995; Wood et al., 2001). There is also evidence for Ca^{2+} in regulation of the movement of the leaves in legumes. Several legumes exhibit movements of their leaves such as *R. pseudoacacia* and *Albizzia lophantha*, circadian rhythms of leaf movement were proved to be maintained by Ca^{2+} (Gómez and Simón, 1995; Moysset et al., 1994).

5) *Hormone Production and Responsiveness*

Recently, several studies on the plant growth regulators have indicated that additional complication of circadian regulation can be no longer ignored. Ethylene production exhibits circadian rhythm in a number of species: barley, wheat and rye (Ievinsh and Kreicbergs, 1992), *Chenopodium rubrum* (Machackova et al., 1997) and *sorghum* (Finlayson et al., 1998; Finlayson et al., 1999; Morgan et al., 1997). In *sorghum*, the

plant showed the rhythm in ethylene production reflects underlying rhythms in mRNA abundance for the ACO2 gene encoding 1-aminocyclopropane-1-carboxylic acid oxidase and in ACC oxidase activity (Finlayson et al., 1999; Schaffer et al., 2001) The other plant growth regulator gibberellin (GA) showed a diurnal rhythm biosynthesis in *sorghum* (Foster and Morgan, 1995). It is likely that more hormones will exhibit circadian rhythms in production.

The circadian oscillators

Circadian rhythms are generated by a central network of 6-12 genes which form interlocked transcriptional feedback loops (Dunlap, 1999; Iwasaki and Kondo, 2000; Young, 1998). Three loops have been identified at present: morning loop, evening loop, and the core loop which interacts between morning and evening loop (Pokhilko et al., 2012). The morning-acting two single MYB domain transcription factors CLOCK-ASSOCIATED (CCA1) and LATE ELONGATED HYPOCOTYL (LHY) as negative repressors, together with positived-activator (TIMING OF CAB EXPRESSION1) TOC1 also known as PSEUDO-RESPONSE REGULATOR 1 (PRR1) which works in the evening, form the central circadian feedback loop in *Arabidopsis* (Alabadí et al., 2001; Matsushika et al., 2000; McClung, 2006; Pokhilko et al., 2012). The CCA1/LHY proteins bind directly to the TOC1 promoter and inhibit its expression (Alabadí et al., 2001) The expression of LHY/CCA1 is also regulated by transcriptional co-regulators PRR9, PRR7 and PRR5/NI (PSEUDO-RESPONSE REGULATORs 9, 7, 5/night inhibitor) (Farré et al., 2005; Nakamichi et al., 2010). They altogether form the so-called morning loop (Farré et al., 2005; Nakamichi et al., 2005; Salomé and McClung, 2005). The evening loop was represented by TOC1, which inhibited the expressions of EC (evening complex) genes including EARALY FLOWERING4 (ELF4), ELF3 and LUX ARRHYTHMO (LUX)/PHYTOCLOCK1 (Pokhilko et al., 2012).

As an important component of evening loop, ELF3 is considered to be involved in the transmission of light signals to the clock (McWatters et al., 2000; Pokhilko et al., 2012), which are involved in the circadian timing and are defining components of the light input pathway. *elf3* mutants are arrhythmic when under constant light condition but not in dark condition (Covington et al., 2001; Hicks et al., 2001; Hicks et al., 1996). Elf3 oscillates with the same phase as TOC1, and activate CCA1/LHY (McWatters et al., 2000). In addition, ELF3 and PHYB interact in the yeast two-hybrid assay. Therefore, ELF3 as opposed to a component of a central oscillator can modulate light inputs to the clock by light (Liu et al., 2001).

1.3 The effect of light on plant

All organisms respond to environmental signals and modify their behaviour or development. Light is the most important ones among these signals which was studied widely in plant with various life activities. It is not only as a source of energy but also as a stimulus that regulates numerous developmental and metabolic processes, from seed germination to the onset of flowering. Light also can affect the clock through activating various photoreceptors (Somers et al., 1998a). The circadian clock can be reset by light has been reported. Crosthwaite found that the *Neurospora* clock could be reset by light through an induction of FREQUENCY (FRQ) transcription (Crosthwaite et al., 1997; Crosthwaite et al., 1995). In *Drosophila*, light resets the clock through degradation of TIM protein (Naidoo et al., 1999; Young, 1998). The circadian clock is entrained by multiple light photoreceptors including the red/far-red PHYTOCHROMES (PHYs) and the blue light photoreceptors CRYPTOCHROMES (CRYs) (Yanovsky and Kay, 2001) as well as interaction among them. Four classes of photoreceptors have been identified in *Arabidopsis*, but the list is not completed. It includes three UV-A/blue light receptors: 1) phototropin, a photoreceptor to sense light direction; 2) two cryptochromes (cry1 and cry2) that mediate many photomorphogenic responses (Briggs and Huala, 1999; Cashmore et al., 1999) and 3) five phytochromes (phyA-phyE) that absorb mainly red/far-red light red and far-red light (600-800 nm), with phyA also responding to broad-spectrum light (UV-A to far-red) of very low intensity and low intensity of blue light (Quail et al., 1995); 4) and Zeitlupes (ZTL, FKF1, and LKP2) (Christie, 2007; Demarsy and Fankhauser, 2009; Lin and Shalitin, 2003; Somers and Fujiwara, 2009).

Generally, photoreceptors are chromoproteins consisting of an apo-protein combined with a variety of chromophores (Christie et al., 1998; Imaizumi et al., 2003; Lin et al., 1995; Rockwell et al., 2006). The

characteristic absorption spectra of chromophores and photoreceptors are simplify presented by Kami (Kami et al., 2010).

1.3.1 Phototropins

Phototropins (phot1 and phot2) specifically induced by UV-A/blue light (320-500 nm), are involved in regulating light-dependent processes that contribute to optimize the photosynthetic efficiency of plants and promote growth (Briggs and Christie, 2002; Celaya and Liscum, 2005). Initially identified from the *Arabidopsis* mutant nph1 (non-phototropic hypocotyl 1), it was named phototropin thereafter, and then renamed phototropin 1 instead of nph1. In Arabidopsis, they function as photoreceptors for phototropism, light-induced chloroplast movement, and stomatal opening (Jarillo et al., 2001; Kagawa et al., 2001; Sakai et al., 2001).

Phototropin contains two flavin-binding domains-PAS (light-oxygen-voltage 1 (LOV1) and light-oxygen-voltage 2 (LOV2), within its N terminus and a typical Ser/Thr protein kinase domain at the C-terminus (Taylor and Zhulin, 1999). Each phot LOV domain binds FMN non-covalently as a chromophore to form the holoprotein (Briggs and Huala, 1999). Except as the phototropins, the LOV domains also present in two additional types of protein in *Arabidopsis*: 1) ZEITLUPE (ZTL)/FLAVINBINDING, KELCH REPEAT, F-BOX 1 (FKF1)/ LOV KELCH PROTEIN 2 (LKP2) (Nelson et al., 2000; Schultz et al., 2001; Somers et al., 2000) and 2) PAS/LOV protein (also called LOV/LOV protein (LLP)). The ZTL/FKF1/LKP2 proteins are involved in the circadian clock and photoperiodic flowering, whereas the physiological function of PLP is largely unknown (Kasahara et al., 2010; Ogura et al., 2008).

1.3.2 Phytochromes and Cryptochromes

Phytochromes are cytosolically localized dimeric chromopeptides with monomers of 120-130 kDa. They own two photoconvertible forms: the red light-absorbing form (Pr) and far-red light-absorbing form (Pfr). They can interconvert to each other in some light conditions (Kay et al., 1989). The earliest and simplest hypothesis of phytochrome action was that responses are triggered by a red light pulse, converting biologically Inactive Pr can convert to active Pfr when illuminating by a red light pulse. Conversely, the process can be reversed by a subsequent brief irradiation with far-red light, converting Pfr back to Pr. In *Arabidopsis*, five phytochromes are encoded by divergent genes (Clack et al., 1994; Sharrock and Quail, 1989).

PhyB is the major red light receptor which plays a role in seedling de-etiolation, light-regulated cell elongation, shade avoidance, and the regulation of flowering time by day length (Reed et al., 1993). PhyD and phyE mutants have more subtle phenotypes that are only revealed in double or triple mutant combinations (Devlin et al., 1998; Devlin et al., 1999; Whitelam and Devlin, 1997). PhyA, is the only light-labile type phy in *Arabidopsis*, plays a major role in gene expression and germination, and mediates very low fluences of broad spectrum light into circadian clock (Botto et al., 1996; Hamazato et al., 1997; Johnson et al., 1994; Shinomura et al., 1996). PhyA is also essential for de-etiolation in far-red enriched light (Dehesh et al., 1993; Nagatani et al., 1993; Whitelam et al., 1993). Collectively, type I and type II phys play distinct roles in live, but their role can be overlapping, coordinated, or even antagonistic (Parks and Spalding, 1999; Reed et al., 1994; Shinomura et al., 2000; Smith et al., 1997; Somers et al., 1998a)

Plant cryptochromes, specifically mediate responses to blue light, showing a strong absorption peak in the blue region of the spectrum, which are receptors for blue and ultraviolet (UV-A) light (400-500 nm).

Cryptochromes were first discovered in *Arabidopsis* at the molecular level through the isolation of the HY4 gene, now commonly referred to as cry1 (Ahmad and Cashmore, 1993). They were first discovered in the plants *Arabidopsis thaliana* and *Sinapis alba* one decade ago, and afterwards identified not only in many other plant species but also in animals, humans, and bacteria. Therefore, cryptochromes (are ubiquitous UV-A/blue light receptors of prokaryotes and eukaryotes.

These photoreceptors show significant homology to microbial photolyases but lack photorepair activity. At least cry1 is a 75-kDa protein that binds both a flavin and a pterin chromophore. The levels of cry2 are reduced by increasing irradiances of blue light but are not affected by red light (Lin et al., 1998). This is not the case with cry1. In *Drosophila*, cryptochrome appears to be the only photoreceptor involved in light input to the clock, and residual effects of light on rhythmic behavior are considered to be the indirect consequence of vision effects on behavioral rhythms (Emery et al., 2000). Cryptochrome physically interacts with clock components (Ceriani et al., 1999). In mammals, the situation has been more controversial. Cryptochromes were first proposed as photoreceptors involved in the light input to the circadian clock (Miyamoto and Sancar, 1998). In plants, cry1 and cry2 are clearly important for normal control of growth and development by blue light (Devlin and Kay, 2000). Both cry1 and cry2 are the blue and ultraviolet-A light receptors, but cry2 is degraded at high fluence rates and cry1 dominate blue light receptor. Mutations on these genes increase the period of rhythmic expression of a photosynthetic gene under certain fluence rates of blue light (Devlin and Kay, 2000; Somers et al., 1998a). The double cry1 cry2 mutant retains robust rhythmicity in *Arabidopsis*, indicating that in contrast to the situation in mammals, cryptochromes are not essential components of the clock (Devlin and Kay, 2000; Yanovsky et al., 2000).

Photoreceptors play key role in mediating light input to clock. PhyA and cry1 can transmit low-fluence blue light to the clock (Somers et al., 1998a). PhyA and phyB as well as cry1 and cry2 play to maintain circadian period length (Millar et al., 1995; Somers et al., 1998a). There are some other blue light receptors including phototropin (NPH1) and ZEITLUPE (ZTL). Recently, ZTL is involved in the length of circadian period by controlling TIMING OF CAB EXPRESSION 1 (TOC1) which is the proteasome-dependent degradation of a central clock protein (Jarillo et al., 2001; Kim et al., 2007; Más et al., 2003; Somers et al., 2000).

Each of the photoreceptor families is composed of members specialized for high and low light responses despite owning similar absorption properties. For instance, phyA, cry2, and phot1 specialized for low light, but phyB-E, cry1, and phot2 function in high light (Lin et al., 1998; Sakai et al., 2001; Smith and Whitelam, 1990).

Chapter 2 Documenting and datamation of process in flower opening and closure

2.1 Data collected in flower of *E. grandiflorm*

Digital cameras (Optio WG-2, Ricoh Imaging, Tokyo) were fix in front of the flowers such that the position of the distal tip of one petal can been seen clearly, and the flower images from the side view were taken automatically at 60-minute intervals for four consecutive days or at 2-minutes intervals to see the movement of petals at dawn and dusk. The flower images were displayed on the computer screen and the relative position of the petal distal tip as compared with the flower base (the boundary between the peduncle and receptacle) along the direction of flower axis (*H*, Fig.2-1) was measured as pixels using MB-ruler version 5.0 software (Markus-Bader, Iffezheim, Germany). The relative position of the distal tip was recorded for one petal from each flower for four consecutive days. Then the normalized relative position of the petal distal tip (*Y*) was calculated as:

$$Y = H_t / H_{max}$$

, where H_t represent H at the time of t, H_{max} is the maximum of H during the four consecutive days for each flower (Fig. 2-1). After the calculation of Y values for each flower, the average of Y values of more than three independent flowers was calculated for each time points.

The oscillation period was estimated by manually finding the peak Y values for each flower opening and closure cycle, and calculating the average intervals between the times for these peak Y values for each flower. The estimated oscillation periods were averaged for at least three independent measurements, and given as means ± standard deviations.

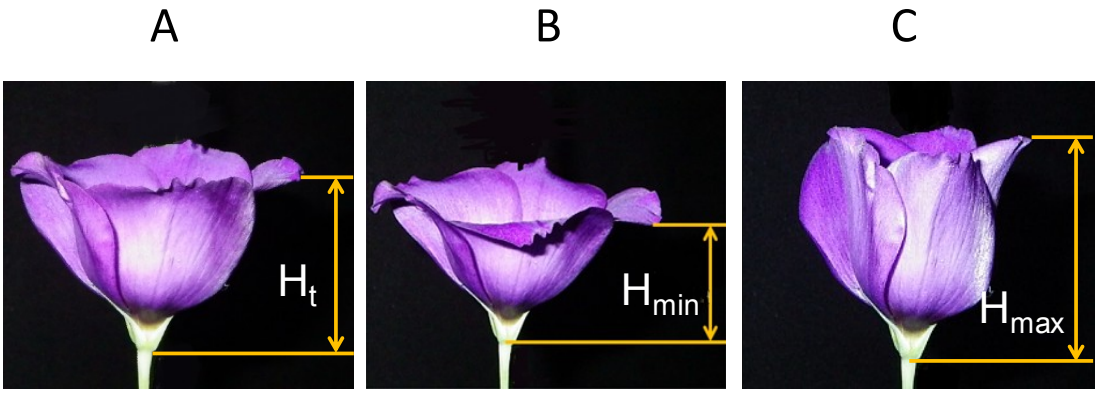

Fig. 2-1. The flower opening and closure process was recorded as the movement of the position of the petal distal tip from the base of the flower. The measured position at time t (H_t) was normalized as $Y = H_t / H_{max}$, where H_{max} represent the maximum of H_t. A: Intermediate stage of flower opening and closure; B: Fully opened flower; C: Fully closed flower.

2.2 Data collected in flower of tomato

Data collection in tomato is the same as *E. grandiflorum*, but not in calculation. The relative position of the petal distal end (*Y2*) was calculated as

$$Y2 = (H_{max} - H_t) / H_{max} \text{.....Eq. 1.}$$

, where vector H_t represent H at the time of t, vector H_{max} is the maximum of H during the two consecutive days for each flower (Fig. 2-2). After the calculation of *Y2* values for each flower, the average of *Y* values of more than three independent flowers was calculated for each time point.

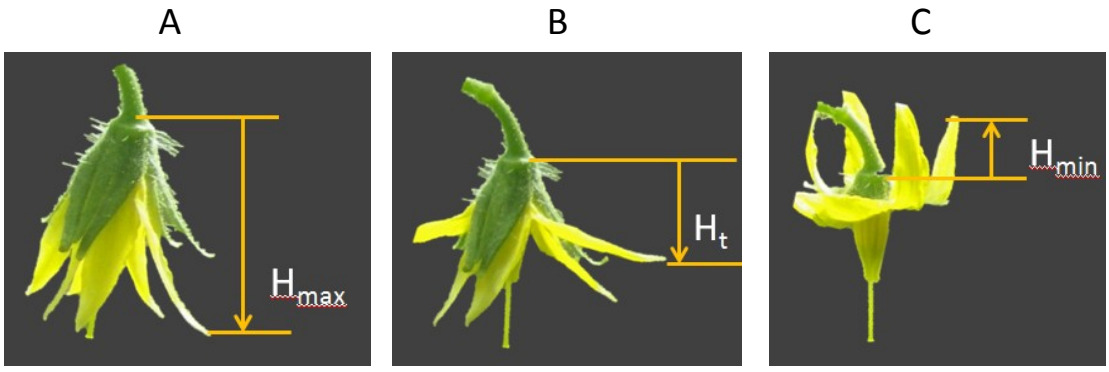

Fig. 2-2. The flower opening and closure process was recorded as the movement of the position of the petal distal tip from the base of the flower (H_t) in tomato. The relative position was calculated as Y2 = (H_{max} -H_t)/ H_{max}, where vector H_t represent value of H at the time of t, vector H_{max} is the value of maximum of H. A: Intermediate stage of flower opening and closure; B: Fully opened flower; C: Fully closed flower.

Chapter 3 Regulation of diurnal rhythms of flower opening and closure by light cycles, wavelength, and intensity in *E. grandiflorum*

Abstract

Flowers of *E. grandiflorum* open in the morning and close in the evening, showing diurnal rhythms. In this study, the process of flower opening and closure of *E. grandiflorum* "Azuma-no-Murasaki" were examined under different light cycles by capturing corolla images by interval photographing. At 24-hour light cycles, the flower opening rhythm synchronized with the light cycles, and the process was composed of dual steps. The first one was immediate opening and closure after dawn and dusk, respectively. The second one was gradual opening and closure, which occurred 12 hours after the end of former light period and 2-3 hours after the initiation of current light period, respectively. The first response appeared to be a direct effect of lights, while the second one appeared to be under the regulation of circadian clocks. Under constant dark, blue, or red conditions, flower showed circadian oscillations of 25.5 ± 0.6 h, 25.6 ± 0.6, or 24.3 ± 0.4 h, respectively. Under the constant white light or co-irradiation of blue and red light, flower opened and closed once, but the oscillations did not continue thereafter. The synchronization of flower opening and closure rhythms to 24- and 20-hour day cycles was observed both for blue light and red light cycles. The synchronization was not complete for 16-hour light cycles and the flower oscillation period became 24 hours under 12-hour light cycles. The direct effect of light was found to be dependent on light intensity. When blue light intensity was adjusted at 25, 40, or 100W·m^{-2}, flower opened more sharply after dawn at a stronger light intensity, but such intensity dependent effect was not observed for red light.

3.1 Introduction

The rhythm of flower opening and closure has been studies since more than a century ago. Many flowers open in the morning and start to close before the end of light period (Bünning and Zimmer, 1962; Ewusie and Quaye, 1977). Therefore, fully open flower cannot be maintained just by keeping lightings on. In most cases, flowers open and close responding to circadian clocks. Circadian clock regulated flower opening was studied in detail for *Pharbitis* (Kaihara and Takimoto, 1979; Kaihara and Takimoto, 1980). The *Pharbitis* flower opening was not regulated directly by light, but was initiated 10 hours after the onset of darkness at 24 °C. Therefore, when the dark period was less than 10 hours, flowers opened during the light period, whereas, if the dark period was longer than 10 hours, flowers opened during the dark period. Flowers of *Turnera ulmifolia* also started to open a certain period after the end of previous light period (Ball, 1933).

As compared with circadian clock regulated flower opening, circadian clock regulated flower closure has been paid less attention and thus is less understood. The duration of floral opening period is usually studied in consideration of floral longevity, which is associated with pollination and subsequent senescence. Significant shorting of floral longevity after pollination can be observed in many species (Niu et al., 2011; Primack, 1985; Van Doorn, 1997). However, many flowers show repeated flower opening and closure and the oscillation seems to be under the regulation of circadian clocks.

E. grandiflorum is one of the flowers that show reciprocal oscillations of flower opening and closure. The purpose of this study was to investigate flower opening and closure rhythms synchronized with environmental light rhythms for establishing the basis for controlling and extending the period of fully open status of the flowers. We designed different light-dark cycles to examine the control of flower opening and closure by lights in *E. grandiflorum*.

3.2 Material and methods

3.2.1 Plant materials

Seeds of *E. grandiflorum* 'Azuma-no-Murasaki' (Sakata Seed, Yokohama, Japan) were sown on wetted peat moss based medium and placed in a growth chamber controlled at 20 °C with 16 hour light / 8h dark light cycles at light intensity of 5 W m^{-2} by fluorescent lamps. Two months after germination, the seedlings were transplanted to 15 cm plastic pots filled with a 3:1 mixture of granular soil and a peat-based soil mix, and grown in a glasshouse for two months until flower buds became visible. About one week prior to the experiments, the plants were transferred to a growth room with the light cycles of 16 hour light period at 25°C and 8 hour dark period at 20 °C, relative air humidity of 60-80%, and light intensity of 100 W m^{-2} over the canopy from the metal halide lamps.

3.2.2 Methods

Experiment 1 - Effects of shading leaves and flowers under light-dark cycles on flower opening and closing rhythms

Before flowers start to open, plants were transferred to light cycles of 16L8D light-dark cycle light period, leaves and flowers were covered by opaque box respectively (Fig. 3-1). The light intensity and temperature of are the same as condition 16L8D, which described in materials. The flower images were recorded from the first cycle of flower opening and closure.

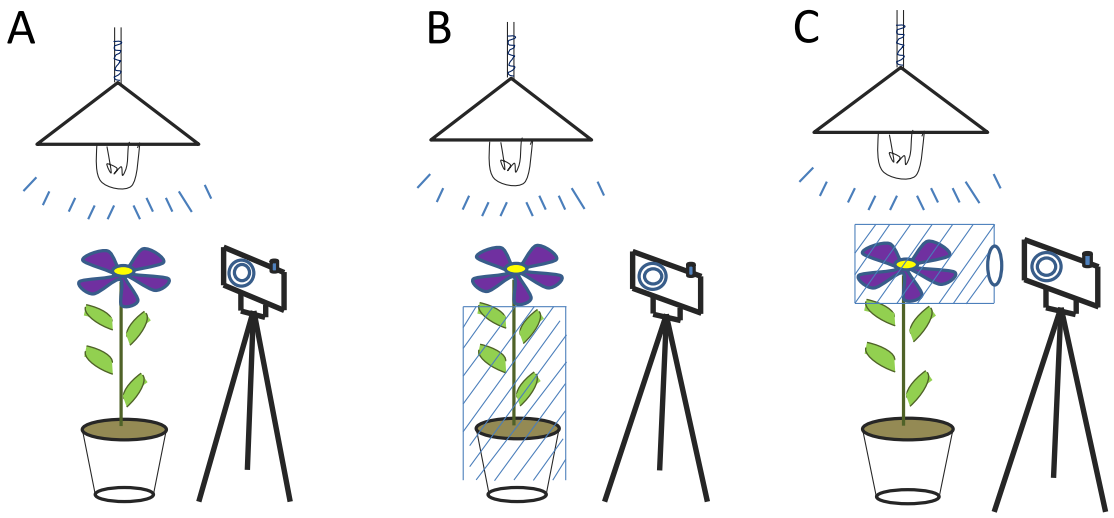

Fig. 3-1. The simple diagram of leaves and flower shading experiment under 16L/8D (light/dark) cycles.A: Flowers without shading; B: Leaves were coved by opaque box; C: Flowers were coved by opaque box.

Experiment 2 - Effects of different light periods under 24-hour light cycles on flower opening rhythms

Plants similarly prepared as in Experiment 1 and placed at light cycles of 20, 16, 12, 8, or 4-hour light period under 24-hour day length (20L4D, 16L8D, 12L12D, 8L16D, 4L20D, respectively). The light intensity and temperature of these treatments are the same as condition 16L8D, which described in materials. The flower images were recorded from the first cycle of flower opening and closure. Other plants were kept under 16L8D conditions until the first day of flower opening, and then subjected to continuous dark or light conditions from the end of the last 16L8D cycle. The flower images were recorded from the beginning of DD and LL conditions.

Experiment 3 – Effect of blue and red light cycles on flower opening rhythms

Plants similarly prepared as in Experiment 1 and placed under the 16L8D condition were transferred to a dark room controlled at 25 °C and subjected to following light treatments from the end of the last 16L8D condition, using blue (OSB56A5111A, Optosupply, 465-475nm) or red (OSR7CA511A, Optosupply, 650-670nm) LEDs: 1) different day-length cycles of 16, 12, 8, or 4-hour light period and 8 hour dark period (16L8D, 12L8D, 8L8D, 4L8D, respectively), using blue (40 $W \cdot m^{-2}$) or red (50 $W \cdot m^{-2}$) light, 2) continuous blue (40 $W \cdot m^{-2}$), red (50 $W \cdot m^{-2}$), or blue plus red lights, 3) 16L8D light cycles using different intensity of blue (25, 40, or 100 $W \cdot m^{-2}$) or red (25 or 70 $W \cdot m^{-2}$) light.

3.3 Results

3.3.1 Effects of part organs shading on flower opening and closing rhythms

When the flowers opened for the first time at 16L8D, petal unfolding of the tightly closed buds proceeded very slowly during the light period, but once flowers fully opened, flowers always started to open within 15 minutes after dawn, and kept rapid opening for 2-4 hours (Fig. 3-2). After this rapid opening, flowers gradually closed until the dusk, and started to close rapidly within 5 minutes after dusk (Fig. 3-2). Since the flower-opening pattern was different between the day of the first flower opening and the following days, the first flower-opening day was regarded as the "preceding day" (Fig. 3-2) and excluded from the measurement of diurnal flower rhythms. The rapid flower opening at dawn was not clear for the preceding day.

Similar as 16L8D condition, at leaf-shading treatment, flower also exhibited opening and closure oscillations synchronized to the light cycles (Fig. 3-3). Flower started to open rapidly, flower started to close after reaching full opening. It showed that flower closed slowly until light-off. Flower closed very fast at 15-20 minutes, then keep closing until dawn. By contrast, at flower-shading treatment, flower exhibited opening and closure rhythms with an oscillation period of 24 ± 0.3 h. However, there was no violent movement on petal, the trends showed smoothly. At this treatment, amplitudes decreased from the second day-night cycle. The amplitudes of the first cycle is three times of the fourth cycle (Fig. 3-3). From the results of fig 3-3, we found that the flowers of *E. grandiflorum* is sensitive to light rather that leaves. Thus, we illuminated the flowers when under the different light wavelength.

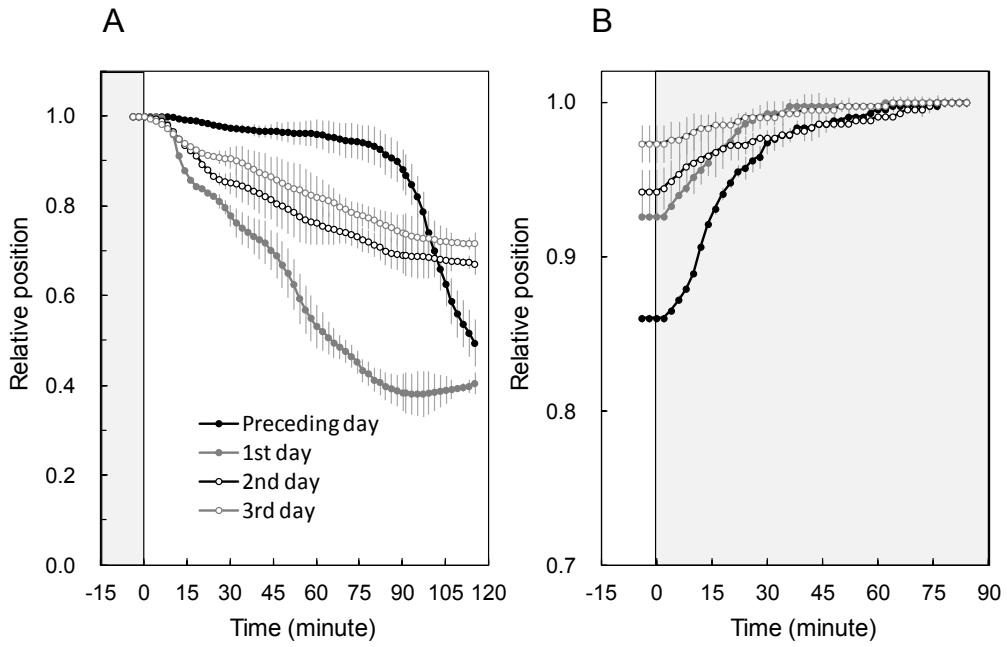

Fig. 3-2. The movement of petals after the transition of the light condition from dark to light (A) and from light to dark (B). The petal movement was recorded during the day of first flower opening ("preceding day") and subsequent three consecutive days. Grey and white areas of each figure indicate dark and light period, respectively. Bars indicate the standard deviations (n = 3).

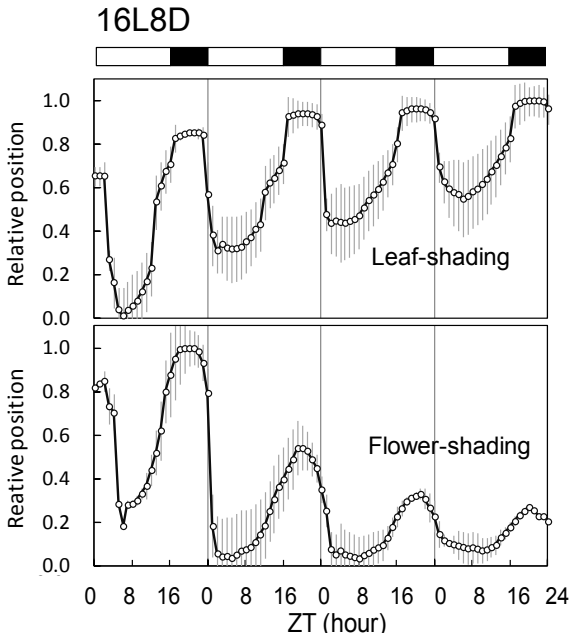

Fig. 3-3. Effects of partial organs shading under 24-hour light cycles on flower opening and closure rhythms. Upper (green line) and Lower (red line) are the data under leaf-shading and flower-shading condition, respectively. Each value is the mean of at least three replicates. Bars indicate the standard deviations ($n \geq 3$).

3.3.2 Effects of different light periods under 24-hour light cycles on flower opening rhythms

At 12L12D light cycles (Fig. 3-4C), flowers also started to open immediately after dawn, reaching the maximum in 2 to 4 hours, closed gradually until dusk, and then closed rapidly immediately after the dusk.

At shorter light period cycles (8L16D, 4L20D), flower opening started during the dark period approximately 12 hours after dusk (Fig. 3-4D,E). Flowers also exhibited rapid opening at dawn and rapid closure at dusk similarly to the cycles with shorter dark periods.

In the longest light period cycles (20L4D), flowers opened only slightly at dawn. The extent of maximum opening became less as flowers repeated daily opening and closure cycles at longer light period cycles (20L4D, 16L8D, 12L12D), so that the *Y* value drifted upward toward the end of the measurement.

Under continuous dark conditions (Fig. 3-5A), flowers exhibited opening and closure rhythms with an oscillation period of 25.5 ± 0.6 h. Under the continuous light (Fig. 3-5B), flowers opened and closed once as observed under the light period of light/dark cycles, and opened again around 24 hours after the beginning of the light period, but they did not show any more opening and closure thereafter.

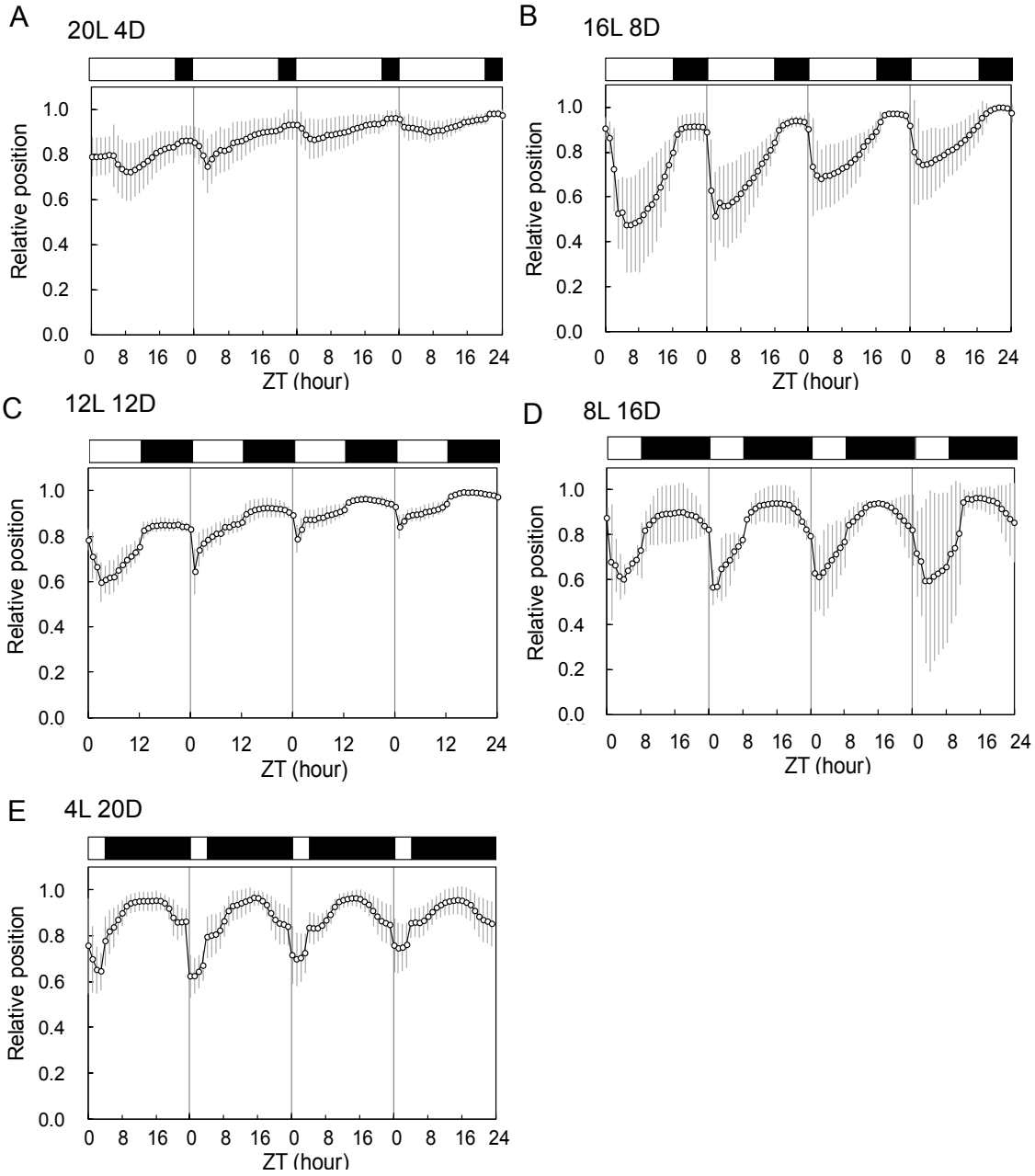

Fig. 3-4. Effects of the length of light periods under 24-hour light cycles on flower opening and closure rhythms. White and black bars above the figures indicate light and dark periods. A-E: Flower opening and closing oscillation rhythm in 24hour light periods (20L4D, 16L8D, 12L12D, 8L16D and 4L20D respectively). Each value is the mean of at least three replicates. Bars indicate the standard deviations ($n \geq 3$).

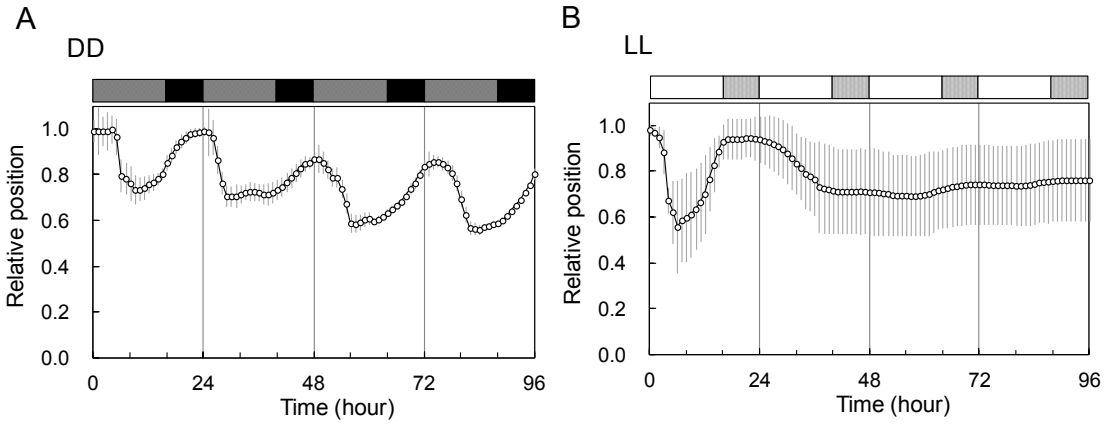

Fig. 3-5. Effects of the length of light periods under constant conditions on flower opening and closure rhythms. The periods indicated by grey bars in LL (continuous light) means the corresponding time of the day during the pre-experimental light condition was dark. A-B: Flower opening and closing oscillation rhythm in constant dark and light period respectively. Each value is the mean of at least three replicates. Bars indicate the standard deviations ($n \geq 3$).

3.3.3 Effect of blue and red light cycles on flower opening rhythms

Under 16L8D and 12L8D light cycles using blue or red light (Fig. 3-6), the flower also exhibited opening and closure oscillations synchronized to the light cycles. The periods oscillation are 24.2 ± 0.3 hours and 20.3 ± 0.3 hours in 16L8D and 12L8D light cycles, respectively. In red 16L8D and 12L8D cycles, they showed 23.9 ± 0.1 hours and 20.1 ± 0.2 hours respectively. The synchronization was not complete for 16-hour light cycles (8L8D), where flowers exhibited around 24-hour oscillation for three cycles by red light or irregular movements by blue light. Under 4L8D light cycles, flower opening rhythm was synchronized to 24-hour cycles both for the blue light and red light, the periods are 24.3 ± 0.4 hours and 23.8 ± 0.6 hours in blue and red of 4L8D light cycles, respectively.

Under the constant blue or red light (Fig. 2-8), flower opening and closure showed free running oscillations; the period of the cycle was 25.6 ± 0.6 hours for blue and 24.3 ± 0.4 hours for red. Under constant co-irradiation of blue and red light (Red + Blue), flower movements were similar to those under constant white light; flower exhibited opening and closure once, but did not show any more rhythms thereafter.

To see the light intensity response of flower opening and closure, flowers were subjected to 16L8D rhythm of blue or red light at different light intensities (Fig. 3-8). The flowers exhibited the sharpest response at dawn to the strongest blue light, and the flowers closed slowly at lower blue light intensities. By contrast, such sharp changes at dawn were not observed for red at both weak and strong intensities. At weak red light cycles, petals opened more widely, as represented by the lower values of Y at full opening, and did not close fully, resulting in downward shift of Y.

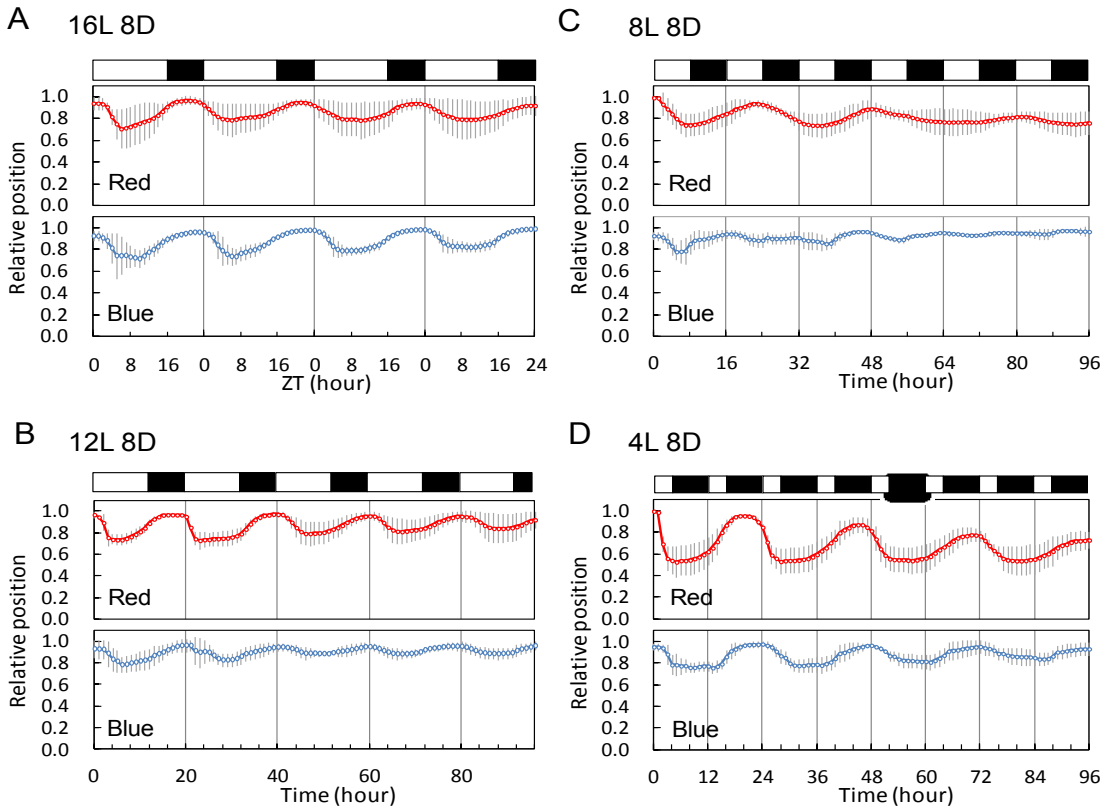

Fig. 3-6. Effects of day length on flower opening and closure under red (upper figures) or blue (lower figures) light cycles. The length of dark period was fix at 8 hours. White and black bars above the figures indicate light and dark periods. A-D: Flower opening and closing oscillation rhythm in different red or blue light cycles (16L8D, 12L8D, 8L8D and 4L8D respectively). Each value is the mean of at least three replicates. Bars indicate the standard deviations ($n \geq 3$).

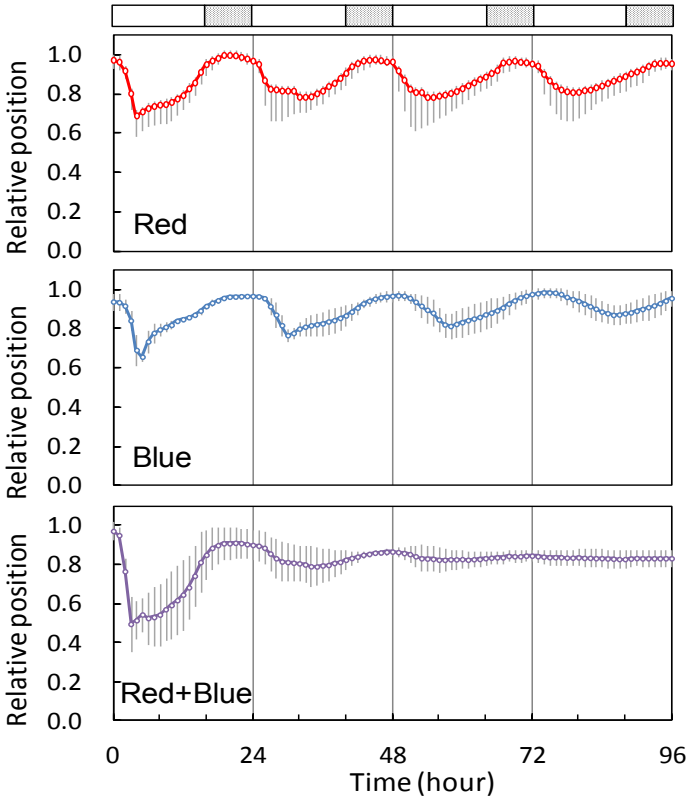

Fig. 3-7. Effect of continuous irradiation of red (upper) or blue (middle), or co-irradiation of red and blue (bottom) on flower opening and closure. The periods indicated by grey bars means the corresponding time of the day during the pre-experimental light condition was dark. Each value is the mean of more than three replicates. Bars indicate the standard deviations ($n \geq 3$).

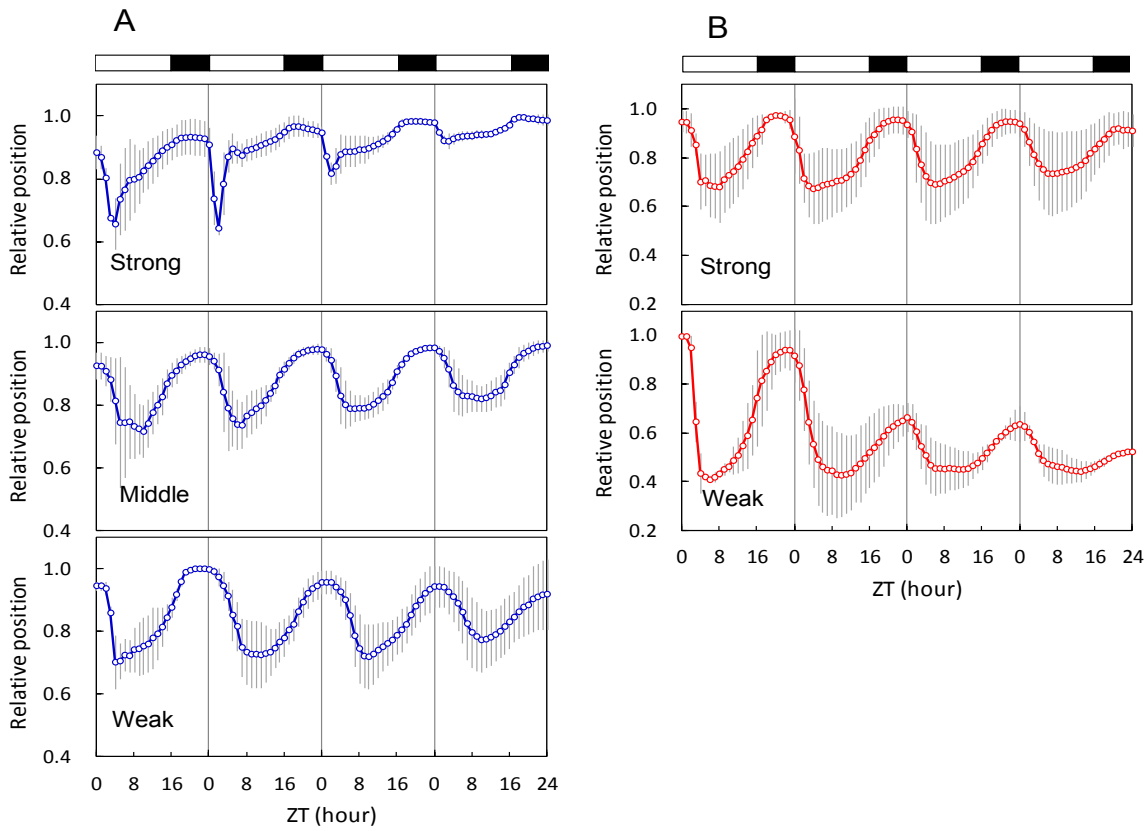

Fig. 3-8. Flower opening and closing oscillation rhythm in different blue light-intensity (A) and in different red light-intensity (B). Light intensity of blue was 100 (Strong), 40 (Middle), and 25 (Weak) $W \cdot m^{-2}$, and that of red was 75 (Strong) and 25 (Weak) $W \cdot m^{-2}$. Each value is the mean of more than three replicates. Bars indicate the standard deviations ($n \geq 3$)

3.4 Discussion

3.4.1 Dual step regulated flower opening and closure

Flowers of many species show reciprocal opening and closure synchronized with environmental light rhythms. This process is usually slow and takes hours for full opening and closure (Tanaka et al., 1989). In this study, the petal movement during flower opening and closure was recorded by interval photographing using digital cameras.

At the standard cycle of 16L8D, flowers opened shortly after dawn, and start to close 2-4 hours after the initiation of light period, showing 24 h period oscillations. Careful observation of the flower opening process revealed that flower opening was composed of dual steps: one started at dawn, and the other started 12 hours after dusk. When the dark period was longer than 12 hours, flowers partially opened during the dark period, and rapidly open immediately after dawn. Similarly, flower closure started during the light period, and flowers immediately closed at dusk. The former dark period opening can be considered as circadian clock regulated movement and the latter one would be a direct and immediate effect of lights. Such a dual system was proposed for once flower opening of Asiatic lily, in which petals opened to approx. 40° in a ‘dark phase’, and opened further for full opening in a subsequent "light phase" (Bieleski et al., 2000b).

3.4.2 Circadian clock regulated flower opening

As described above, the flower opening started during the dark period before dawn when the dark period was longer than 12 hours (8L16D and 4L20D), and flower closure started during the light period when the light period was longer than 4 hours. The dark period flower opening and light period flower closure suggested the involvement of circadian clocks in the regulation, since these changes required substantial periods of hours after the actual changes in light conditions. To confirm this, persistence of flower opening rhythms under constant dark or light conditions was tested.

The flowers showed an approximately 25-hour rhythm at least for three days in DD (Fig. 3-5), indicating the involvement of circadian clock in *E. grandiflorum* flowers.

In contrast to the continuous dark condition, being placed in the continuous light condition, flowers opened and closed once, and showed no more oscillation of flower opening and closure thereafter. Thus more than minimum dark period seems to be required for the flowers to open. The requirement of a stretch of dark period for flower opening was reported for other plants. The minimum time requirement was 7 h for Asiatic lily flower (Bieleski et al., 2000b) and a few hours for *Turnea ulmiflolia* (Ball, 1933). In *E. grandiflorum* flowers, flowers showed only slight opening and closure at 20L4D cycles. Thus, minimum requirement of dark period would be around 4 hours in *E. grandiflorum* flowers.

3.4.3 Direct and immediate effect of lights on flower opening and closure

The *E. grandiflorum* flowers exhibited rapid opening within less than 15min after the initiation of light period (Fig. 3-2A). Rapid flower opening after dawn has been reported for other plants before. It took less than one hour for *Portulaca* (Ichimura and Suto, 1998), less than 20 min for *Oenothera biennis* (Sigmond, 1930) and only 5 min for *Hedera helix* (Sigmond, 1929) to reach its full flower opening after the initiation of illumination. Rapid flower closure was also observed at the beginning of dark periods (Fig. 3-2B). These immediate responses to the light phase transition were observed regardless of different light/dark cycles and even in shorter day length cycles (Fig. 3-4B, C, D). Therefore, it is likely that the immediate response is the direct effect of the transition of light condition.

The immediate flower opening after dawn appears to be dependent on both light quality and light intensity. Under the blue/dark cycles with the strongest blue light, flowers exhibited the sharpest changes at the point of light on and off (Fig. 3-6A), while the light intensity dependent response was not clear under the red light cycles (Fig. 3-6B). In excised leaves of

Oxalis corymbosa, leaf movement, which was directly induced by light, was found to be mediated by blue light, but not by red light (Nakanishi et al., 2005). It is generally found that the extent of flower opening depends on environmental light intensities. On cloudy days at low sunlight intensities, flowers often show insufficient flower opening. Based on our results, blue light may play an important role in these light-intensity dependent flower openings. Although many studies showed the association of flower opening with light intensity as reviewed previously (van Doorn and van Meeteren, 2003), the physiological basis for this response remains unknown.

3.4.4 Flower opening and closing rhythm controlled by multiple photoreceptors

Our data showed that red light or blue light was sufficient for the synchronization of flower opening and closure rhythms (Fig. 3-6). The flower opening oscillation synchronized with both red/dark and blue/dark cycles of different day length. The circadian rhythm was also observed under continuous blue and continuous red as in DD (Fig. 3-7, 3-5A). However, when both red and blue were irradiated continuously, the circadian rhythm disappeared as observed for LL (Fig. 3-7, 3-5B). Therefore, signals of multiple photoreceptors would be integrated, and the integrated signals may control the flower opening and closure rhythms. Red/far-red-light photoreceptor phytochromes and blue-light photoreceptor cryptochromes were found to play a key role in the synchronization of circadian oscillations to light/dark cycles (Mas and Yanovsky, 2009; Somers et al., 1998a; Yanovsky and Kay, 2001) In *Pharbitis*, the time of flower opening was delayed by red irradiation during the dark period, and the effect could be cancelled by subsequent far-red irradiation (Kaihara and Takimoto, 1979; Kaihara and Takimoto, 1980), suggesting the involvement of red/far-red reversible phyB in the regulation. From our data, both

cryptochromes and phytochromes seem to be involved, but the involvement of other photoreceptors for blue light would be possible.

3.4.5 Molecular mechanisms for circadian clock regulation of flower opening and closure

The circadian free running under DD but not under LL were similar to the flower opening rhythm of roses (Horibe and Yamada, 2014), but different from some other plants. *Bellis perennis* flower showed a circadian rhythm under LL, while *Kalanchoe blossfeldinana* shows circadian rhythm under both under LL and DD (Karvé et al., 1961). In *Arabidopsis*, leaf movement and hypocotyl elongation oscillated in LL after LD pretreatment, but the oscillation was not clear under DD.

Our understanding of circadian clock regulation is too limited to speculate why different species showed different response to LL and DD, but these variations in the response to continuous light conditions would be related to the entrainment mechanism of circadian clocks. The absence of oscillation under LL suggests that the oscillation of endogenous clock was disturbed or output of clock signals were interrupted by the continuous light signal. In *Arabidopsis*, even though the oscillation disappeared under DD, the transcripts levels of *LATE ELONGATED HYPOCOTYL* (*LHY*), *CIRCADIAN CLOCK ASSOCIATED 1* (*CCA1*), *GIGANTEA* (*GI*), *and EARLY FLOWERING 4* (*ELF4*) genes, which were discovered to control internal rhythms, continued to exhibit circadian rhythms under both LL and DD following the light/dark cycle (Doyle et al., 2002; Higuchi et al., 2011; Schaffer et al., 1998; Wang and Tobin, 1998), while the expression of *TIMING OF CAB EXPRESSION 1* (*TOC1*) showed oscillation in LL, but not in DD (Strayer et al., 2000). These data implicated that circadian oscillators were active under LL and DD in *Arabidopsis*, but the irregular output signals under DD caused the arrhythmic behavior of the leaves. Accordingly, the loss of oscillation of *E. grandiflorum* flowers under continuous LL would be resulted from the defects in the output pathway of

oscillator genes, rather than arrhythmicity of oscillators. To clarify these relationships, it would be necessary to analyze the oscillations of clock genes in *E. grandiflorum*.

The observed immediate response upon the transition of light conditions appears to be the “acute response”, which represents the “gating” of clock output signals (Millar and Kay, 1996). The morning genes are usually induced by light exposure, and this entrains circadian clocks to the environmental light rhythms. However, the extent of the response is not stable throughout the day, but shows circadian rhythms. When the response is small, the “gate” is regarded as being closed. Therefore, the entrainment of the rhythm can be completed effectively only during the specific periods of the day.

In this regard, the immediate response upon the transition of light conditions should be influenced by the manner of the light/dark cycles. In fact, the immediate response was not clear for 20L4D, and in this condition, the flower opening and closure rhythm was disturbed. Similarly, at 4L8D cycles, no immediate response was observed and the period of flower opening and closure was not 12 hours, but 24 hours. These examples may imply that the gate was closed at dawn in these conditions so that the rhythm could not be entrained to the light cycles.

3.5 Conclusion

E. grandiflorum flower showed 24-hour cycles of flower opening and closure, and this cycle was precisely synchronized to environmental light/dark cycles. The rhythms could be synchronized both by blue and red light, indicating that both red photoreceptor such as phytochromes and blue light photoreceptors such as cryptochromes were involved in the entrainment of the rhythm. The data also showed that the flower opening and closure was controlled by a dual system, one is controlled by circadian clock, and the other functions immediately after the light was on and off. Flower opening gradually started approximately 12 hours after the start of dark period, and rapidly after the light-dark phase changes. Blue light seemed to be especially important for the light-intensity dependent regulation of flower opening and closure.

Chapter 4 The effect of red/far-red in diurnal opening and closure in flowers of *E. grandiflorum*

In this chapter, we investigated the effect of red light and/or far-red light on *E. grandiflorum* 'Azuma-no-Murasaki' under different light cycles by capturing corolla images by interval photographing. The flower showed normal opening and closing rhythm under different light conditions. The circadian amplitudes are obviously decreased in far-red /dark condition but not in red/dark and far-red red/dark conditions. Flowers exhibited differed response on condition of revering the red and far-red light. Flower showed the second opening in condition of 8Fr8R8D whereas no evident change in 8R8Fr8D condition. We also perform the far-red light-break during the light period to investigate the role of red and far-red light on flower opening and closing rhythm. The amplitude of oscillation obviously reduced from 0.19 to 0.09 in the condition of 14R1Fr1R8D. However, in condition of 15R1Fr8D, it was almost invariable till the fourth day.

4.1 Introduction

Among external environmental factors, light is the most important factor influencing plant development at all phases of its life cycle (Kircher et al., 1999; Neff and Chory, 1998). As light photoreceptors, phytochromes (absorb red and far-red light) and cryptochromes (absorb strongly blue and UV-A light) were wildly study on photosynthetic activities, leaf movement and gene expression (Millar, 1999; Somers, 1999). Recently, they mediated light signal input to circadian clock to regulate life activity have been reported (Devlin and Kay, 2001; Finlayson et al., 1998; Halliday and Fankhauser, 2003; Kendrick and Kronenberg, 1994; Yanovsky et al., 2001). The most extensively researched photoreceptors are phytochromes, which regulate gene expression by switching between the red-absorbing form (P_r) and the far-red absorbing form (P_{fr}). That is because P_r is the biologically inactive form which is easy to converts to P_{fr}, whereas P_{fr} is the biologically active within plants which controls transcription level of various genes (Batschauer, 1999; Chalker - Scott, 1999; Ni et al., 1999).

There are a number of studies about light-break also known as light perturbation on the floral initiation of plants (Goto et al., 1991; Vince-Prue, 1983; Vince-Prue, 1994). Generally, red light-break is effective on inhibiting floral initiation in long dark period of short-day plant. This effect will be eliminated by exposure to brief far-red light (Downs, 1956). However, this effect will be reversed in long-day plant. That is, light-beak cloud promotes floral initiation in long-day plant (Evans et al., 1965; Friend, 1968a; Imhoff et al., 1979). FR is supposed can cause the enhancement of floral initiation though the "hight irradiance response" of phytochrome (Deitzer et al., 1979; Friend, 1968b).

In this chapter, we performed different light period condition with red light and far-red light to investigate the exact roles of them in regulation of flower diurnal opening and closing rhythm.

4.2 Material and methods

4.2.1 Plant materials

Plant materials and growing conditions are the same as in chapter 2.

4.2.2 Methods

Before flowers start to open, plants were transferred to a dark room controlled at 25 °C and subjected to following light treatments from the end of the last 16L8D condition, using far-red (710-740 nm) or red (OSR7CA511A, Optosupply, 650-670nm) LEDs:

1) 16L8D light cycles with far-red light (80 $W \cdot m^{-2}$).

2) Continuous far-red (80 $W \cdot m^{-2}$), and far-red plus red lights.

3) 16L8D light cycles (red/far-red conversion): a) the previous 8 hours is red light (70 $W \cdot m^{-2}$), and the next 8 hours is far-red light, then 8 hours dark; b) the previous 8 hours is far-red light (70 $W \cdot m^{-2}$), and the next 8 hours is red light, then 8 hours dark; c) the previous 15 hours is red light (70 $W \cdot m^{-2}$), and last 1hour is far red light; d) the previous 14 hours is red light (70 $W \cdot m^{-2}$), and the next 1hour is far-red light, the last 1 hour of light period is red light, then 8 hours dark.

4.3 Results

4.3.1 Effect of far-red light on flower opening and closing rhythm

To find out which light or photoreceptor is cure factor in flower opening and closing rhythm in *E. grandiflorum*, we also carried the plant to far-red conditions. Under the condition of far-red/dark cycles (16L8D), flowers opened rapidly when illumined by far-red light, then keep state of full opening till closed to dusk, flowers started to close at around ZT15-16 (Fig. 4-1). The flowers continue to close during the dark period till dawn. The amplitude of oscillation decreased from the second day. Under constant far-red light condition, the flowers also keep normal oscillation rhythm with approximately 24 hours photoperiod. Similar to condition of far-red/dark cycles (16L8D), the amplitude decreased below one third. We also performed the red plus far-red/dark 16L8D light condition, the flowers opened rapidly at dawn, then maintain full opening till at time of ZT 12-13, they did not closed when enter dark period, this phenomenon lasted for several days. In addition, the amplitude have no obvious change in red plus far-red/dark 16L8D light condition.

Far-red plus red light 16L8D conditions were also performed, flowers opened at dawn in 4-5 hours then closed slowly till dusk, flower closed completely. Notably, the amplitude of oscillation in this condition was almost no change from 2nd day compared with condition of far-red/dark cycles (16L8D). It suggested that red-light play an important role in regulation of circadian amplitude.

Under constant far-red light condition, flowers kept rhythm of opening and closure, but the amplitude decreased obviously. The length of photoperiod was shortened by 22.1 ± 0.3 hours in 2nd day and 21.6 ± 0.5 hours in 3rd day. Up to 4th day, flowers opened at subject dawn, but they did not show closure thereafter.

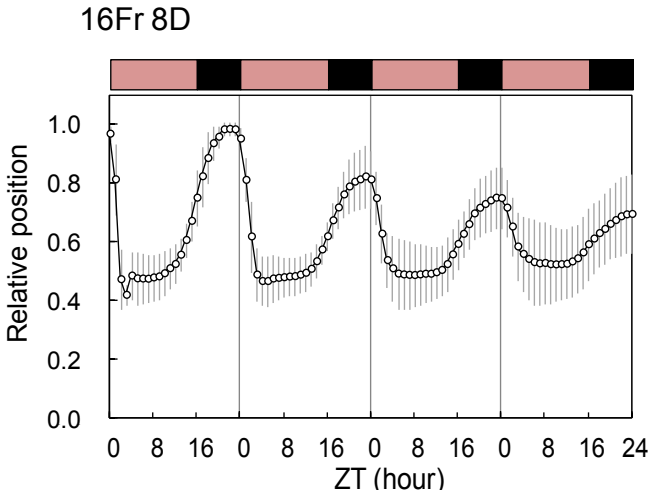

Fig. 4-1 Oscillation rhythm of flower opening and closure under far-red/dark light cycles. Pink and black bars above the figures indicate light and dark periods. Each value is the mean of more than three replicates. Bars indicate the standard deviations (n ≥ 3)

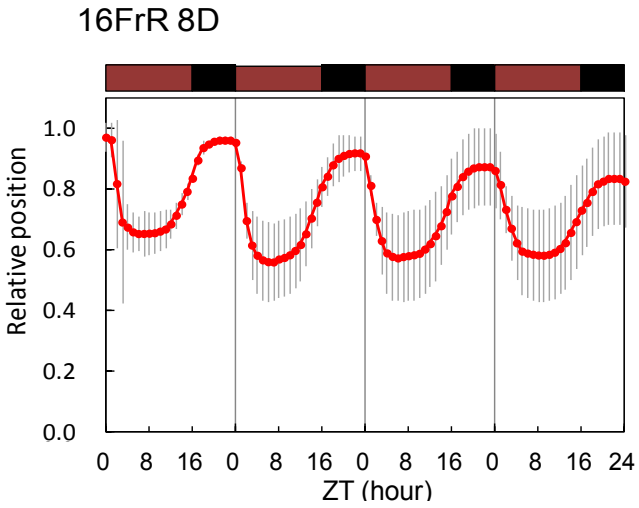

Fig. 4-2 Oscillation rhythm of flower opening and closure under red+far-red/dark light cycles. Dark red and black bars above the figures indicate light and dark periods. Each value is the mean of more than three replicates. Bars indicate the standard deviations (n ≥ 3)

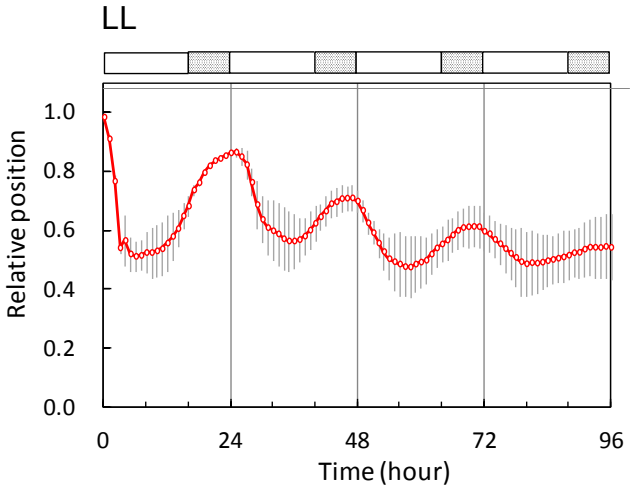

Fig.4-3 Oscillation rhythm of flower opening and closure under constant far-red light condition. The periods indicated by grey bars in LL (continuous far red light) means the corresponding time of the day during the pre-experimental light condition was dark. Each value is the mean of more than three replicates. Bars indicate the standard deviations ($n \geq 3$)

4.3.2 Effect of alternation of far-red/red light on flower opening and closing rhythm

To clarify the roles of red and far-red in flower opening and closing rhythm, we conversed the red and far-red in 16L8D condition. That is previous 8 hours is red light, then 8 hours in far-red light, continuing 8 hours dark (Fig. 4-5). The other experiment is conversion of red and far-red light, that is, previous 8 hours is far-red light, then 8 hours in red light (Fig. 4-4). The flowers showed the second opening in condition of 8Fr8R8D (Fig. 4-4), especially at 1st and 2nd day, whereas no exhibit in condition of 8R8Fr8D (Fig. 4-5). Under the 8Fr8R8D, flowers opened at dawn in 2-3 hours, then the petal moved slowly, up to change to red light (ZT 8-9), flowers opened rapidly again. Interestingly, we found that flowers started to open during the dark period rather than dawn in 8R8Fr8D condition (Fig. 4-5). The speed of closure during the red light is higher than during the far-red light in middle of light period. It is indicated that the effect of red light is higher than far-red.

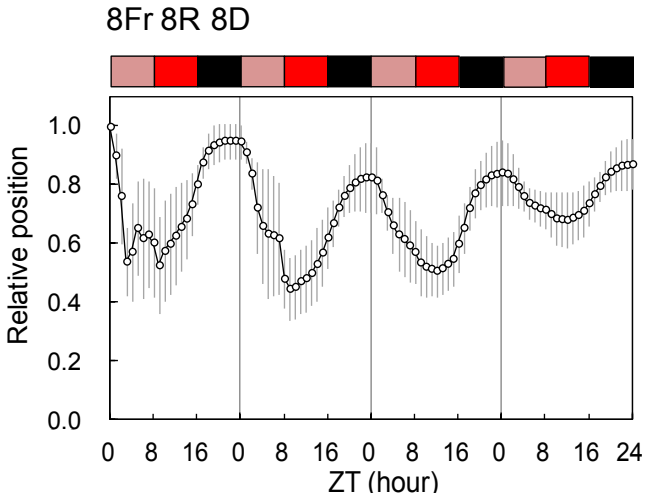

Fig. 4-4. Effects of alternation of red and far-red light on flower opening and closure. Red, pink and black bars above the figures indicate red, far-red and dark periods, respectively. Each value is the mean of more than three replicates. Bars indicate the standard deviations ($n \geq 3$).

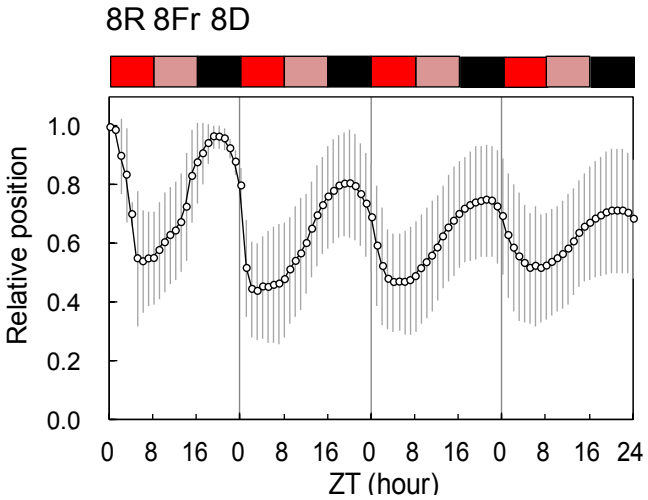

Fig. 4-5. Effects of alternation of red and far-red light on flower opening and closure. Red, pink and black bars above the figures indicate red, far-red and dark periods, respectively. Each value is the mean of more than three replicates. Bars indicate the standard deviations ($n \geq 3$).

4.3.3 Effect of far-red light-break on flower opening and closing rhythm in light period

To find out the exact roles of far-red and red in flower opening and closing, we also performed the far-red light-break near dusk. The details are showed in Fig. 4-6 and Fig. 4-7. Under both two light conditions, flowers persisted opening and closing rhythm, but there are many distinct differences between them. In light condition of 14R1Fr1R8D (Fig. 4-6), the amplitude of oscillation in flower opening and closure decreased day by day, 0.19 in first day, 0.14 in second day, 0.09 in third day and 0.02 in forth day. However, the amplitude of oscillation in flower opening and closure is almost no change in condition of 15R1Fr8D (Fig. 4-7).

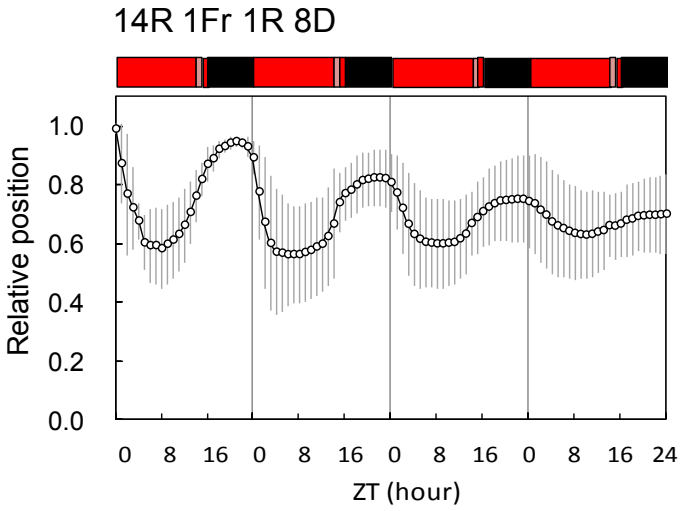

Fig. 4-6. Effects of far-red light broken on flower opening and closure. Red, pink and black bars above the figures indicate red, far-red and dark periods, respectively. Each value is the mean of more than three replicates. Bars indicate the standard deviations ($n \geq 3$).

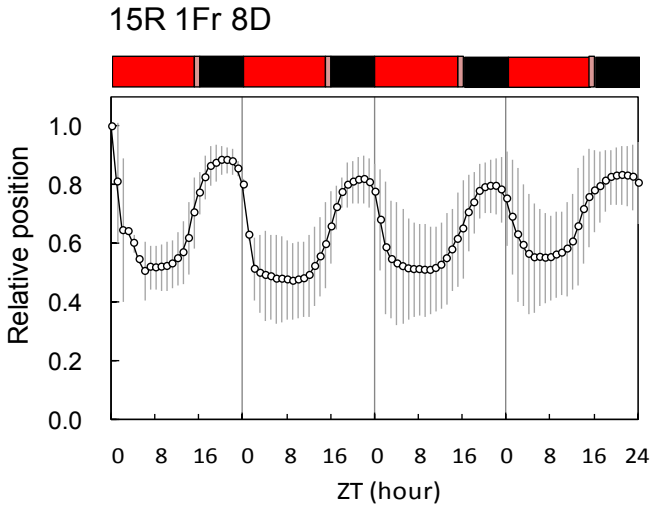

Fig. 4-7. Effects of far-red light broken on flower opening and closure. Red, pink and black bars above the figures indicate red, far-red and dark periods, respectively. Each value is the mean of more than three replicates. Bars indicate the standard deviations ($n \geq 3$).

4.4 Discussion

In this work, results demonstrate the variability in how flower respond to environments with in red, and far-red light. Phytochromes have two interconvertible forms: the P_r and P_{fr} absorbing forms. In long-day plants, flowering is promoted by red light (or high R : FR ratio) during the early part of the photoperiod. Illuminating far-red light (or low R : FR ratio) towards the end of light period or interrupt the dark period promotes extension growth and flowering. (Evans, 1976; Lane et al., 1965; Thomas and Vince-Prue, 1996). Flowering and extension growth can be influenced by R : FR ratios in *V.* ×*wittrockiana*. Under low R : FR ratio condition, stem extension and flowering were promoted, first flowering occurred on the primary stem. On the contrary, branching was promoted and flowering was inhibited under high R : FR ratio condition (Runkle and Heins, 2001).

Under different 16h light (red, far-red and combination of red and far-red) period, flowers showed distinct response (Fig. 4-1, 2 and Fig. 3-6A). The amplitudes of flower opening and closing oscillation were not obviously decreased with illuminating red light or combination of red and red light. Although flowers showed normal opening and closing rhythm in constant far-red light, the amplitude was lower. It inferred that red light is dominant in maintaining the amplitudes of circadian oscillation.

In our study, the flowers showed the second opening in condition of 8Fr8R8D (Fig. 4-4) but not in condition of 8R8Fr8D (Fig. 4-5). Moreover, in condition of 15R1Fr8D (Fig. 4-7), the amplitude of flower opening and closing oscillation can be kept whereas decreasing in 14R1Fr1R8D (Fig. 4-6). It indicated that red light given followed by far-red light during light period overcame the inhibitory effect of far-red light on flowering. It is consistent with that far-red light produces an inhibition of flowering in long day plant. This inhibition can be overcome by subsequent red light. The effect will be reversed in short day plant (Reid et al., 1967).

4.4 Conclusion

E. grandiflorum flower showed opening and closing rhythm in various light conditions of red and far-red light. Red light plays a dominant role in maintaining the amplitudes of circadian oscillations. In the results of light-break experiment, the flowering opening can be promoted by red light. Red light also overcomes the inhibition of flowering which is produced by far-red light.

Chapter 5 Phytochromes and cryptochromes together control the diurnal rhythm of flower opening and closure in *Solanum lycopersicum*

As a popular fruit vegetable plant, tomato (*Solanum esculentum*) is widely studied in physiology, biochemistry, genetics and molecular biology. Tomato flowers open in the morning and close in the evening, showing a diurnal rhythm. With tomato as a model plant, elucidation in flowering physiology in tomato plant is important to understand the mechanisms of plant flowering. In the present study, photoreceptor- deficient mutants of tomato were grown under different light conditions and the diurnal states of flowering were recorded through capturing corolla images by interval photographing. Under continuous light conditions, circadian rhythm persisted in the phyB deficient mutant. In contrast to mutant *phyB2-1*, mutant *phyB1-1* showed obvious oscillation rhythm when under all lighting conditions. In addition, dual mutant of *phyB* deficiency and triple mutant *phyAphyB1-1phyB2-1* exhibited normal oscillations, while rhythm was disappeared in other mutants where *phyB* gene was activated. Under continuous dark conditions, all *cry1*-deficien- mutants showed a shortened length of circadian period. Therefore, it was suggested that red light played a role in mediating the amplitude of oscillations and blue light was related with length of oscillation in circadian clock.

5.1 Introduction

The biological processes including metabolism, physiology and behavior in most living organisms display a endogenous and entrainable diurnal rhythm of approximately 24 h (Dunlap, 1999). For example, the classic system of pulvinar leaf movement showed in leguminous plants (Lumsden and Millar, 1998). This rhythm is driven by swelling of extensor and flexor regions of pulvinus (Kim et al., 1993). There are also circadian rhythms in growth rate and cell elongation. Hypocotyl growth of *Arabidopsis*, exhibiting a circadian rhythm in elongation rate, is regulated by light and hormones (Halliday and Fankhauser, 2003; Nemhauser and Chory, 2002; Symons and Reid, 2003). As an important field in plant science, flower opening and closing rhythms have been widely studied in

many ornamentals (Bünning and Zimmer, 1962; Ewusie and Quaye, 1977; Kaihara and Takimoto, 1979; Kaihara and Takimoto, 1980).

The circadian rhythms rely on molecular oscillators which are controlled by interlocked feedback loops (Wijnen and Young, 2006). Three loops have been identified at present: morning loop, evening loop, and the central loop, which interacts between morning and evening loop (Alabadí et al., 2001; Matsushika et al., 2000; McClung, 2006; Pokhilko et al., 2012; Schaffer et al., 1998; Strayer et al., 2000; Wang and Tobin, 1998). Plant clocks are synchronized by environmental changes (Mas and Yanovsky, 2009), such as through activating various photoreceptors (Somers et al., 1998a). As light photoreceptors, crytochromes absorb blue light (400 to 500 nm wavelength) and phytochromes absorb red/far-red light (600 to 700 nm wavelength) (Christie and Briggs, 2001; Whitelam, 1998). They cater to the synchronization of circadian oscillations (Casal and Yanovsky, 2005; Yanovsky and Kay, 2001). Five phytochromes (phyA to phyB) are identified in *Arabidopsis*. PhyA is light unstable, which dominates in dark-grown conditions and degraded rapidly during the light period. Conversely, other phytochromes are more light stable, and PhyB dominates in light-grown conditions (Kendrick and Kronenberg, 1994; Reed et al., 1993). Remarkably, all phytochromes contain a C-terminal PAS domain, which exists in many clock-associated proteins (PERIOD or TIMELESS) (Bognár et al., 1999; Millar, 1997). Two cryptochromes, cry1 and cry2, are the blue and ultraviolet-A light receptors, which have been characterized in *Arabidopsis,* and mainly act to maintain circadian period length. These photoreceptors are involved in setting the clock and synchronization of circadian oscillations to light-dark cycles (Devlin and Kay, 2000; Somers et al., 1998a; Yanovsky and Kay, 2001).

As a model plant, tomato was widely studied with many mutants in photomorphogenesis (Koornneef and Kendrick, 1994; Van Tuinen et al., 1995). Flower opening and closing oscillation also showed in tomato plants. In order to clarify the relationship between different wavelengths of light and

circadian rhythm, we investigate the response of different mutants to different light conditions.

5.2 Material and methods

5.2.1 Plant materials

Seeds of mutants of tomato (*Solanum esculentum* L. cv. Moneymaker) (Table 1) and a wild type (WT) (*Solanum pimpinellifolium* L.) were sown on seedling plates filled with wetted peat moss in greenhouse. The seedlings were transplanted to 3L plastic pots filled with a 1:3 mixture of a peat-based soil mix (Sakata Seed Co. Ltd., Yokohama, Japan) and granular soil. Plants were grown in a glass house, where the temperature was between 24 and 30 °C and air humidity was between 50% and 80%. The plants were grown for two months until formation of flower buds became visible. Then the plants were transferred to a growth chamber controlled at 25/20 °C (16h light/8hdark), with relative air humidity 60-80%, and light intensity 100 W m^{-2} over the canopy from white lamps. The diurnal regime before the treatments was 16L/8D, similar to the normal in the summer climate in Tokyo area.

5.2.2 Methods

About one week after the plants were placed in the environment-controlled chamber, the flowers started to open. The treatments of lighting regime were started under the fixed 24 h diurnal regimes with different lighting periods as follows: 1) Normal: 16L/8D (16h light/ 8h dark); 2) Continuous light: 24L/0D; 3) Continuous dark: 0L/24D. The light intensity was 100 W m^{-2}.

Table. 1 The name of mutants in *Solanum esculentumm*

Single mutant	**Dual mutants**	**Triple mutants**
phyA	*phyAcry1-1*	*phyAphyB1-1phyB2-1*
phyB1-1	*phyB1-1 phyB2-1*	*phyAphyB1-1cry1-1*
phyB2-1	*phyAphyB1-1*	
cry1	*phyB1-1cry1*	

5.3 Results

5.3.1 The effect of light cycles in conditions of 16L 8D on flower opening and closing in tomato mutants

Under the light regime of 16L 8D, the wild type (*WT*) opened very quick after the beginning of light period, reaching the maximum in two to three h. Flower closure started at ZT10 (about 10 hours after dawn), and closing process was very quickly within the early 2 h (Fig. 5-1). This result showed that the *WT* tomato flowers are sensitive to dark. This movement inopening and closing rhythm can be maintained for several days.

Similar to *WT*, single mutants *phyA*, *phyB1-1*, and *phyB2-1* (Fig. 5-1) and dual mutants *phyAcry1* and *phyB1-1cry1-1* (Fig. 5-2) opened when illumined, then reached the maximum in 2-4 hours. Then, 6 h later, flowers started to close and completely closed at around ZT10, and were also sensitive to dark. However, the mutant *phyB1-1phyB2-1* showed the instability (Fig. 5-2). Unlike other dual mutants, the flowers in the mutant *phyAphyB1-1* opened during the light period and closed around ZT 16.

The diurnal rhythm of opening and closing is observed in two triple mutants *phyAphyB1-1phyB2-1* and *phyAphyB1-1cry1-1* flowers under 16L8D (Fig. 5-3), opening at light-on and closing at ZT 16 (light-off). The results suggested that the flower opening and closing rhythm in plants with mutated *PHYA* and *PHYB1-1* synchronized with the cycle of light-dark.

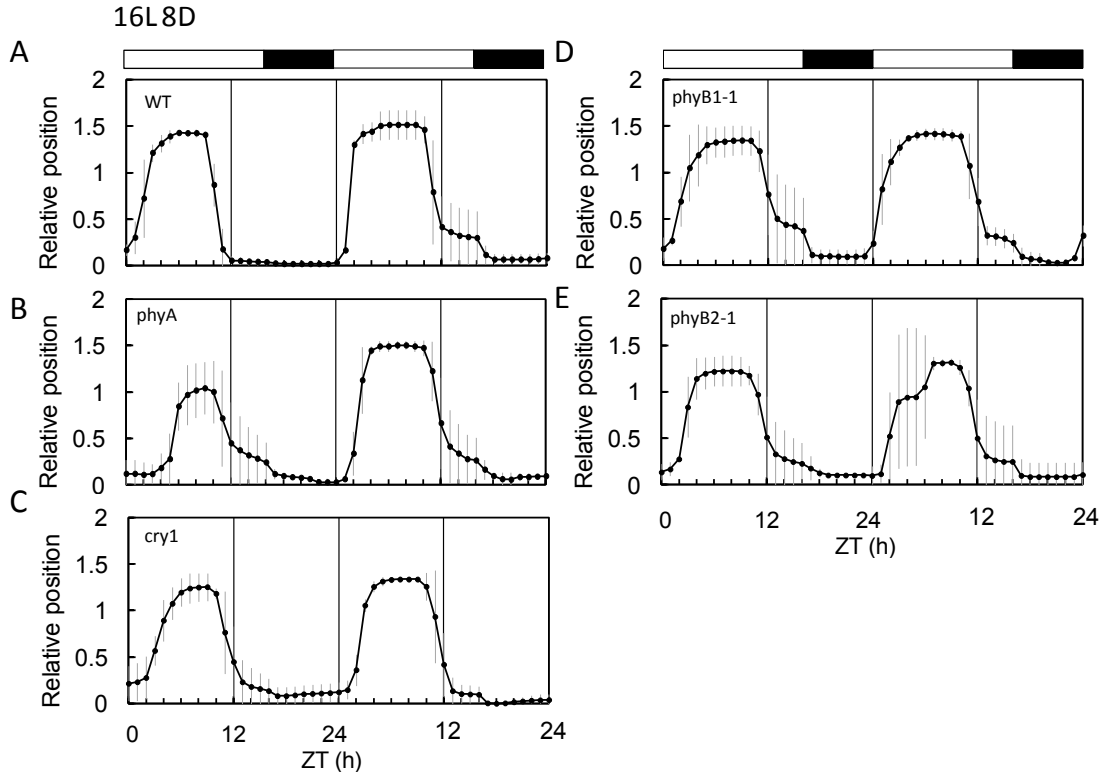

Fig. 5-1 The flowers opening and closing oscillations of single mutants in 16L8D light-dark condition. White and black bars above the figures indicate light and dark periods. A-E: Flower opening and closing oscillation rhythm in different mutants and *WT* (*WT*, *phyA*, *cry1*, *phyB1-1* and *phyB2-1* respectively). Each value is the mean of at least three replicates. Bars indicate the standard deviations ($n \geq 3$).

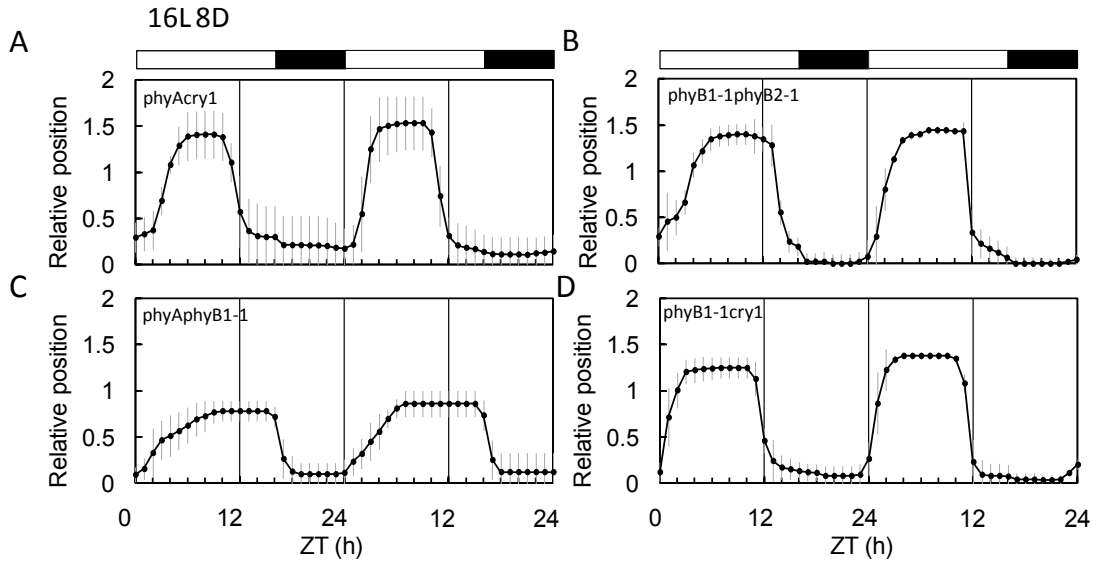

Fig. 5-2 The flowers opening and closing oscillations of dual mutants in 16L8D light-dark condition. White and black bars above the figures indicate light and dark periods. A-D: Flower opening and closing oscillation rhythm in different dual mutants (*phyAcry1*, *phyB1-1phyB2-1, phyAphyB1-1* and *phyB1-1cry1* respectively). Each value is the mean of at least three replicates. Bars indicate the standard deviations ($n \geq 3$).

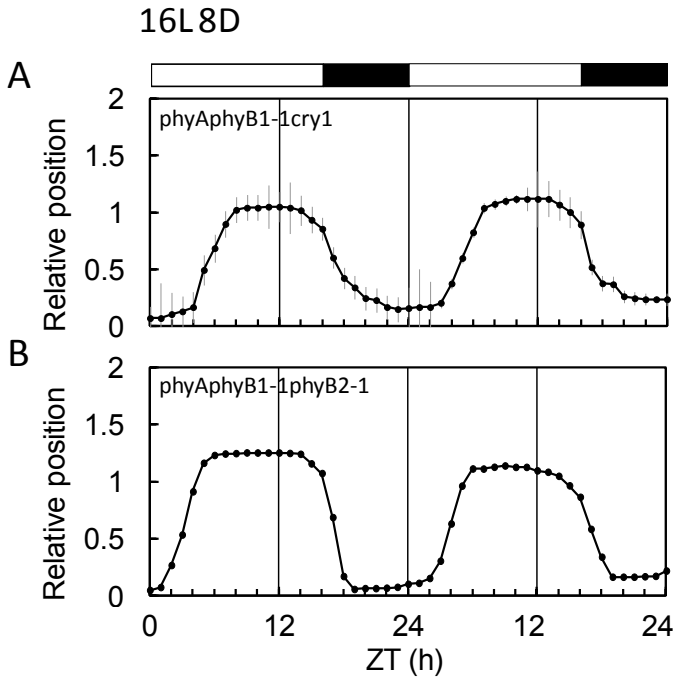

Fig. 5-3 The flowers opening and closing oscillations of triple mutants in 16L 8D light-dark condition. White and black bars above the figures indicate light and dark periods. A-B: Flower opening and closing oscillation rhythm in different triple mutants (*phyAphyB1-1cry1*, and *phyAphyB1-1phyB2-1* respectively). Each value is the mean of at least three replicates. Bars indicate the standard deviations ($n \geq 3$).

5.3.2 The effect of light cycles in conditions of continuous lighting (LL) on flower opening and closing rhythms in tomato mutants

Under continuous light conditions, flowers of *WT* and single mutants *phyA* and *cry1* opened and closed once as observed under the light period of light/dark cycles, then maintained closed, and no more opening and closing oscillation was showed thereafter (Fig. 5-4). However, other single mutants, *phyB1-1* and *phyB2-1*, showed opening and closing rhythms in continuous light conditions. Interestingly, unlike other opening and closing rhythms, the second cycle just showed a brief oscillation in mutant *phyB2-1*.

There are differences among dual mutants with mutated *phyB2-1*. Flowers of mutants *phyAphyB1-1*, *phyB1-1cry1* and *phyB1-1phyB2-1*, with exception of mutant *phyAcry1*, showed diurnal opening and closing oscillations in LL (Fig. 5-5). In addition, the two triple mutants *phyAphyB1-1cry1* and *phyAphyB1-1phyB2-1* persisted oscillations under continuous light conditions (Fig. 5-6). The results showed that the Y value in the dual mutant *phyB1-1phyB2-1* is larger than in the single mutant *phyB1-1*. Combined with the results of *phyB2-1*, we found that the oscillations were observed when mutated gene *PHYB1-1* is present. The function was amplified by mutated allele gene *PHYB1-1* ($Y_{phyB1\text{-}1phyB2\text{-}1} > Y_{phyB1\text{-}1} > Y_{phyB2\text{-}1}$). It is speculated that *PHYB* can hamper the light signal input to circadian clock to protect the endogenous rhythm in tomato.

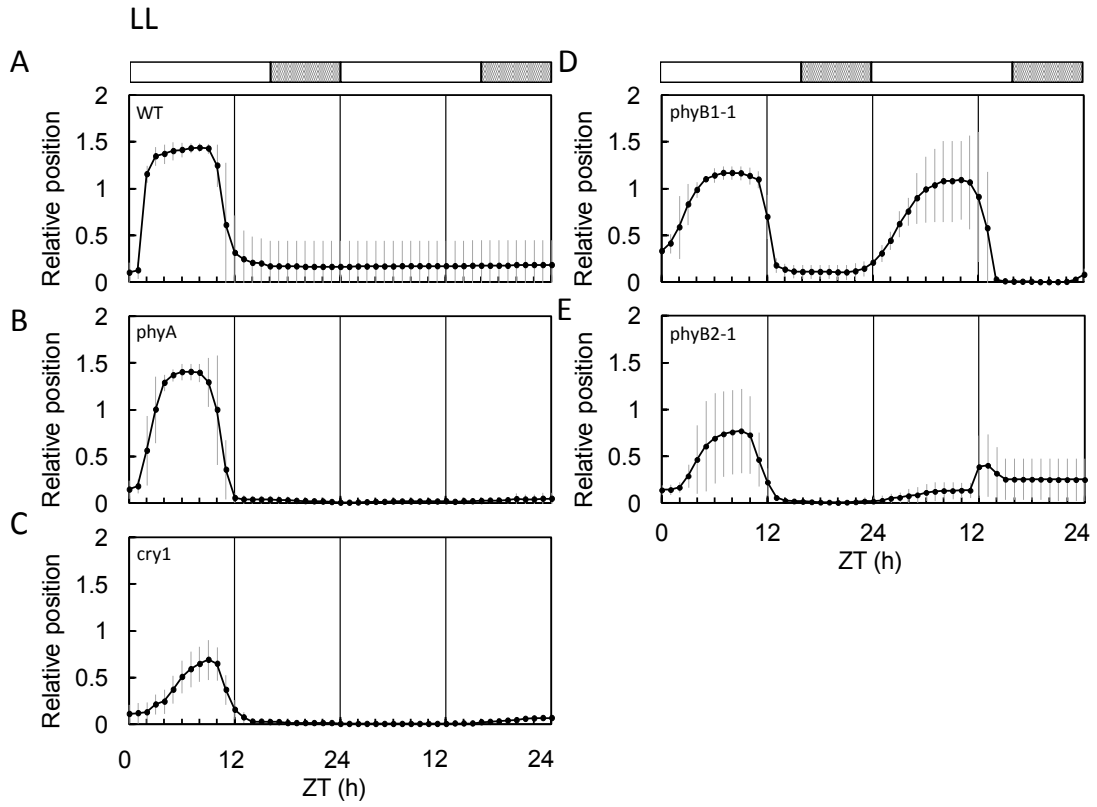

Fig. 5-4 The flowers opening and closing oscillations of single mutants in constant light condition. The periods indicated by grey bars in LL (continuous light) means the corresponding time of the day during the pre-experimental light condition was dark. A-E: Flower opening and closing oscillation rhythm in different mutants and *WT* (*WT*, *phyA*, *cry1*, *phyB1-1* and *phyB2-1* respectively). Each value is the mean of at least three replicates. Bars indicate the standard deviations ($n \geq 3$).

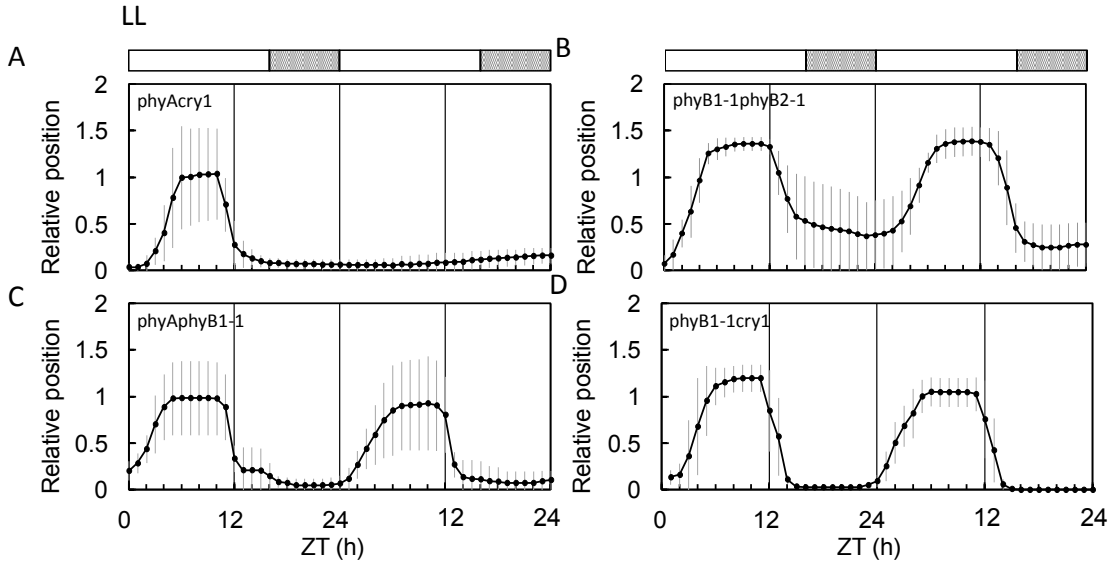

Fig. 5-5 The flowers opening and closing oscillations of dual mutants in constant light condition. The periods indicated by grey bars in LL (continuous light) means the corresponding time of the day during the pre-experimental light condition was dark. A-D: Flower opening and closing oscillation rhythm in different dual mutants (*phyAcry1*, *phyB1-1phyB2-1, phyAphyB1-1* and *phyB1-1cry1* respectively). Each value is the mean of at least three replicates. Bars indicate the standard deviations ($n \geq 3$).

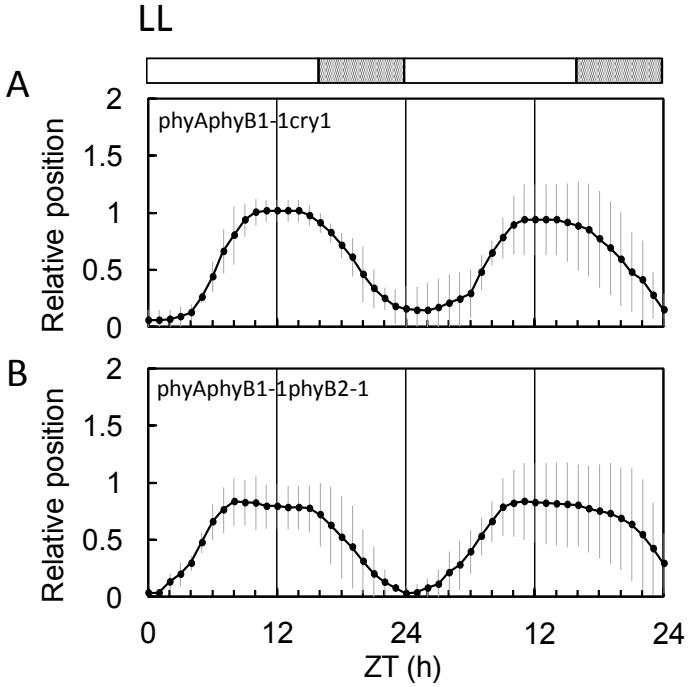

Fig. 5-6 The flowers opening and closing oscillations of triple mutants in constant light condition. The periods indicated by grey bars in LL (continuous light) means the corresponding time of the day during the pre-experimental light condition was dark. A-B: Flower opening and closing oscillation rhythm in different triple mutants (*phyAphyB1-1cry1 and phyAphyB1-1phyB2-1* respectively). Each value is the mean of at least three replicates. Bars indicate the standard deviations ($n \geq 3$).

5.3.3 Effect of light cycles in conditions of continuous dark on flower opening and closing rhythms in dual mutants of tomato

All flowers exhibited opening and closing oscillations under continuous dark conditions, but there are some differences. Under continuous dark conditions, the flower opening and closing rhythm is similar to that in conditions of 16L 8D in *WT*. The flowers of other single mutants spent 5-8 hours to reach the maximum. Flowers of mutant *phyA* closed at time of ZT16 (the time assumed beginning of darkness). After reaching full open, flowers of mutants *phyB1-1*and *cry1* started to close in the second cycle. However, the flowers closed at the time of assumed light-off in mutant *phyB2-1* (Fig. 5-7).

Although both dual mutants *phyAcry1* and *phyB1-1phyB2-1* showed opening and closing oscillations in continuous dark conditions (Fig. 5-8), the values of relative position were lower than that in conditions of 16L 8D (Fig. 5-2). The length of period in mutant *phyB1-1phyB2-1* was around 22 h and shorter than in 16L8D conditions, and the dual mutant *phyAcry1* also showed shorter photoperiod length with about 23 h.

Flowers of all triple mutants showed opening and closing oscillations under 16L8D in continuous dark and continuous light conditions. Under 16L8D and continuous dark, mutants opened at light-on and closed at around ZT16 (light-off), however, the flowers spent much more time to open in continuous condition (Fig. 5-8). Interestingly, the length of periods in continuous dark condition were shortened, about 22h in mutant *phyAphyB1-1phyB2-1* and 23h in mutant *phyAphyB1-1cry1*, however, in continuous light condition, the length of periods was prolonged for 25.5h in mutant *phyAphyB1-1phyB2-1* and 25h in *phyAphyB1-1cry1* (Fig. 5-8).

Interestingly, plants with single mutated *PHYA*, *CRY1* or *PHYA* showed obvious opening and closing oscillation. In addition, mutants with *PHYA* deficiency showed shorter period length of the clock.

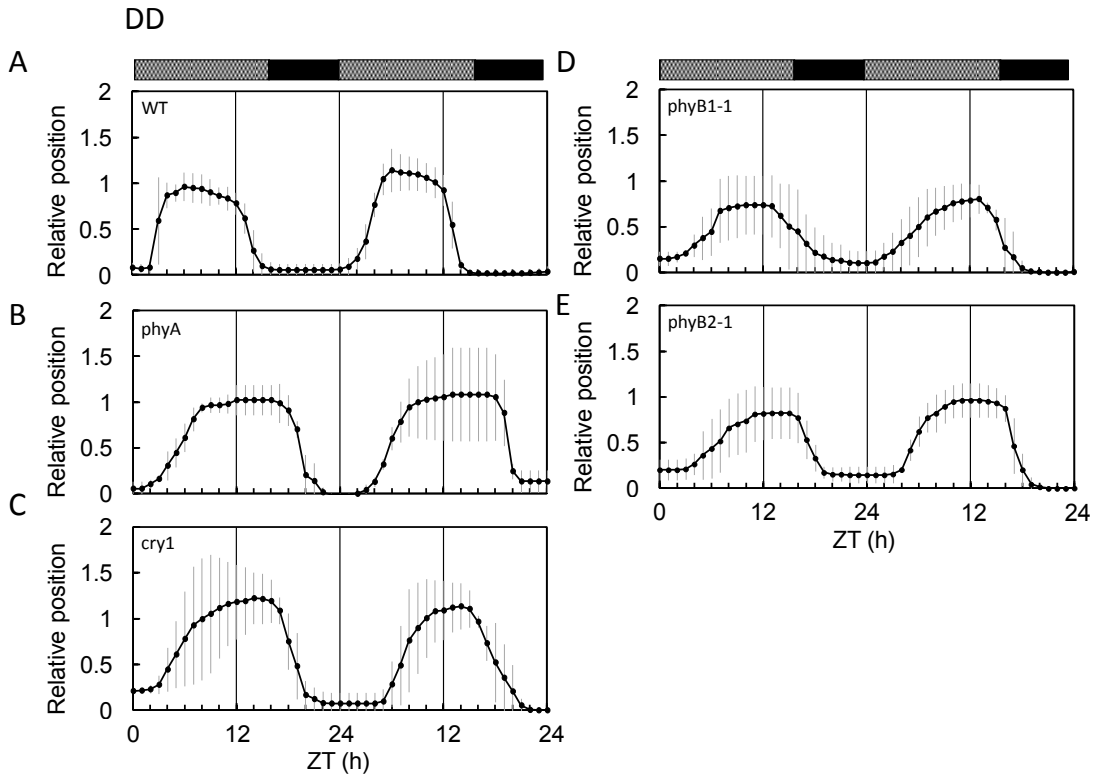

Fig. 5-7 The flowers opening and closing oscillations of single mutants in constant dark condition. A-E: Flower opening and closing oscillation rhythm in different triple mutants (*WT*, *phyA*, *cry1*, *phyB1-1* and *phyB2-1* respectively). Each value is the mean of at least three replicates. Bars indicate the standard deviations ($n \geq 3$).

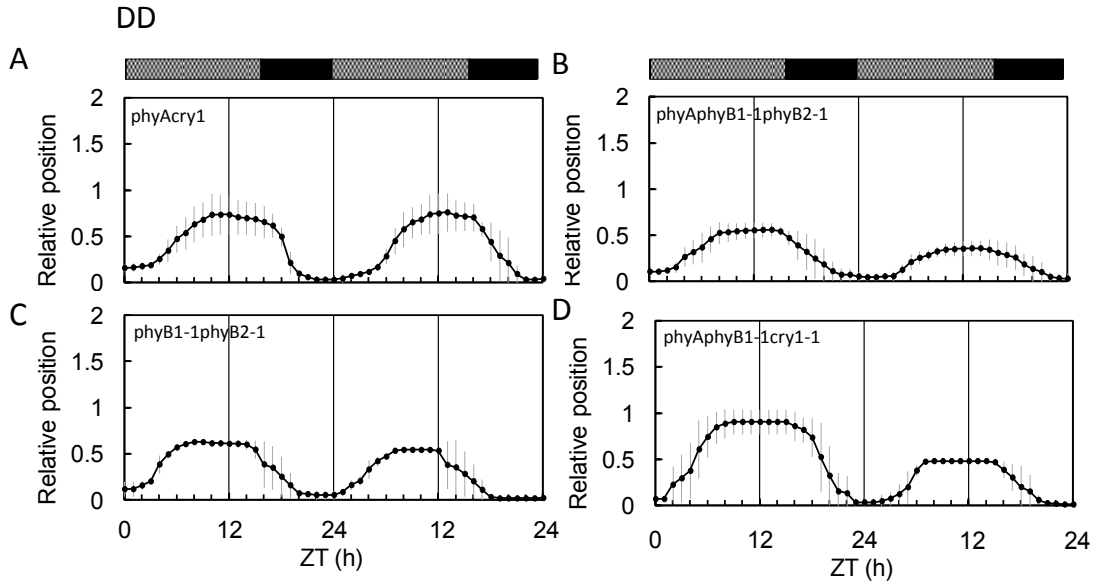

Fig. 5-8 The flower opening and closing oscillations of dual and triple mutants in continuous dark condition. A-D: Flower opening and closing oscillation rhythm in different dual mutants (*phyAcry1*, *phyB1-1phyB2-1, phyAphyB1-1phyB2-1* and *phyAphyB1-1cry1* respectively). Each value is the mean of at least three replicates. Bars indicate the standard deviations ($n \geq 3$).

5.4 Discussion

5.4.1 *PhyB* mutants persisted endogenous circadian rhythm in tomato flowers

In plants, the circadian clock controls daily changes of photosynthetic activities, leaf movement, cell growth flower opening and gene expression (Millar, 1999; Somers, 1999). Blue light photoreceptor cryptochrome and the red/Far-red light photoreceptor phytochrome, as important molecular components have been described for plant circadian systems in *Arabidopsis* (Somers et al., 1998a). In our previous work in *E. grandiflorum*, we found that the amplitude of oscillation decreased in low intensity of red light condition (in chapter 3). Phytochrome B is the primary high-intensity red light photoreceptor for circadian control and phytochrome A acts under low-intensity red light (Somers et al., 1998a). In the results of single mutants *phyB1-1* and *phyB2-1*, flowers opened rapidly at dawn (ZT2) and closed at dusk (around ZT16) in condition of 16L8D. In addition, in continuous light condition, the oscillation rhythms were persistent in both of these two mutants (Fig.5-1, 2, 3, 5, 6, 7). However, flowers of *WT* and mutant *phyA* did not show opening and closing rhythm from the second day when under continuous light condition. Moreover, in dual and triple mutants when phyB-deficient which showed opening and closing rhythms under continuous light condition. Thus, we infer that *PHYB* may be as a inhibitor play a role in mediating light signal input to the circadian clock.

5.4.2 *PHYA* and *CRY1* alter the length of circadian opening and closure in tomato flowers

In many biological organisms, the endogenous oscillator could be entrained to the daily light and temperature cycles of the external environment to maintain rhythms of about 24 hours, to control a wide variety of biological processes (Lumsden and Millar, 1998). Under the condition of continuous dark condition, *E. grandiflorum* flower showed the

oscillation with longer period (25.5 ± 0.6 h). Cry1 is light stable which mediates high-intensity blue light signals to the clock and regulates period length (Somers et al., 1998a). PhyA not only absorb red light to clock but also low-intensity blue light, which is light labile and predominates in dark-grown (Devlin and Kay, 2001). Over-expression of phyB (Wagner et al., 1991) shortened period length by 1 to 3 hours, relative to the *WT*, dependent on the fluence rate (Somers et al., 1998a). In the present study, we found that under continuous dark condition, dual mutants *phyB1-1phyB2-1*, *phyAcry1* and triple mutants *phyAphyB1-1phyB2-1*, *phyAphyB1-1cry1* showed shorter photoperiod length less than 24 hours compared with 16L8D condition (Fig. 5-7, 8). However, the length of photoperiods in flower opening and closing oscillation in mutants *phyAphyB1-1phyB2-1* and *phyAphyB1-1cry1* was prolonged for 25.5 hours and 25 hours respectively in continuous light condition. Collectively, plants with *phyA* deficiency, which showed shorter period length of the clock.

Cry1 as a signal transduction component downstream of phyA with phyA act low fluence rate of blue light input to clock have been known (Ahmad et al., 1998; Lin et al., 1995). In the current study, single mutant *phyA* show approximately 24 hours length of period like *WT* in condition of DD condition, however, shortened in single mutant *cry*1. In addition, we also found the lengths of dual mutant *phyAcry1* and triple mutant *phyAphyB1-1cry1-1* are shortened in DD condition with 23.1 ± 0.3 h hours and 22.3 ± 0.2 hours respectively, but no change in mutants triple mutant *phyAphyB1-1phyB2-1* (24.2 ± 0.3 hours). It is suggested that cry1 located downstream of phyA is playing a role in maintaining the length of circadian clock. (Fig. 5-4, 5, 6, 7, 8). That is, the blue light input to circadian to mediated the length of period.

5.5. Conclusion

Tomato *S. esculentum* flower showed 24-hour cycles of flower opening and closure, and this cycle was precisely synchronized to environmental light/dark cycles. Mutant with phyB-deficient showed opening and closing rhythms in continuous light condition which infer that PHYB may be as an inhibitor play a role in mediating light signal input to the circadian clock. Plants with phyA deficiency, which showed shorter period length of the clock. In addition, the period of dual mutant *phyAcry1* and triple mutant *phyAphyB1-1cry1-1* also are shortened in DD condition. Cry1 located downstream of phyA has been reported (Devlin and Kay, 2000). Thus, phyA and cry1 are playing a role in maintaining the period length of circadian clock.

Chapter 6 Conclusion

Many ornamentals flowers open in the morning and start to close before the end of light period (Bünning and Zimmer, 1962; Ewusie and Quaye, 1977). As ornamentals, which are displayed at flower shops and appreciated by consumers not only during the day but also at night, the ability to keep fully open status of flowers at night or in a specific time of the day is an important trait. For this purpose, development of techniques to control the rhythms or breeding of cultivars that can keep open flowers at any time of the day as consumers prefer would have potential demands.

To date, circadian clock can be reset by light and temperature have been known. Scientists were expecting to find some way to solve the problem that flowers cannot open at certain time. Thus, we investigated the effect of different light condition and photoreceptors genetic defect on flower opening and closing oscillation rhythm in this study.

In chapter 3, the effect of light on flower opening and closing oscillation rhythm was discussed. Flower opening and closing cycle in *E. grandiflorum* was precisely synchronized to environmental light/dark cycles. The rhythms could be synchronized both by blue and red light, indicating that both red and blue light photoreceptors were involved in the entrainment of the rhythm. The data also showed that the flower opening and closure was controlled by circadian clock and the other functions immediately after the light was on and off. Flower opening gradually started approximately 12 hours after the start of dark period, and rapidly after the light-dark phase changes.

In chapter 4, in order to find out the regulating action of red light photoreceptors (phytochromes), we investigated the effect of red/far-red light on flower opening and closure. The data showed that red light is involved in maintaining the amplitudes of circadian oscillations. Red light could overcome the inhibition of flowering which is produced by far-red light.

In chapter 5, we capitalize on mutants of the model plant *Solanum esculentum* whose flowers also show opening and closing oscillation rhythm to define the roles of phytochromes and cryptochromes further. The data indicated that PHYB may act as negative regulators in mediating light signal input to the circadian clock. The data also showed that phyA and cry1 are playing a role in maintaining the period length of circadian clock.

Overall, flower rhythmic opening and closure is mainly controlled by light. As light photoreceptors, PHYs and CRYs play different role in mediating the light input to circadian clock, thereby regulating the rhythmic opening and closing movement. However, there are also many problems remain. Such as our study are just from the perspective of artificial condition in laboratory but in manufacturing and distribution. Thus, study of the ornamentals flowers rhythm opening and closure is to be studied further.

Reference

Ahmad M., Cashmore A.R. (1993) *HY4* gene of *A. thaliana* encodes a protein with characteristics of a blue-light photoreceptor. Nature 366:162-166.

Ahmad M., Jarillo J.A., Smirnova O., Cashmore A.R. (1998) The CRY1 Blue Light Photoreceptor of *Arabidopsis* Interacts with Phytochrome A In Vitro. Molecular cell 1:939-948.

Alabadí D., Oyama T., Yanovsky M.J., Harmon F.G., Mas P., Kay S.A. (2001) Reciprocal regulation between TOC1 and LHY/CCA1 within the Arabidopsis circadian clock. Science 293:880-883.

Ball N.G. (1933) A physiological investigation of the ephemeral flowers of Turnera ulmifolia L. var. elegans Urb. New Phytologist 32:13-13.

Baskin J.M., Baskin C.C. (1976) Effect of photoperiod on germination of Cyperus inflexus seeds. Botanical Gazette:269-273.

Batschauer A. (1999) Light perception in higher plants. Cellular and Molecular Life Sciences CMLS 55:153-166.

Bieleski R., Elgar J., Heyes J. (2000a) Mechanical aspects of rapid flower opening in Asiatic lily. Annals of Botany 86:1175-1183.

Bieleski R., Elgar J., Heyes J., Woolf A. (2000b) Flower opening in Asiatic lily is a rapid process controlled by dark-light cycling. Annals of Botany 86:1169-1174.

Bieleski R.L. (1993) Fructan hydrolysis drives petal expansion in the ephemeral daylily flower. Plant physiology 103:213-219.

Black M., Wareing P. (1954) Photoperiodic control of germination in seed of birch (*Betula pubescens* Ehrh.). Nature 174:705-706.

Bognár L.K., Hall A., Adám É., Thain S.C., Nagy F., Millar A.J. (1999) The circadian clock controls the expression pattern of the circadian input photoreceptor, phytochrome B. Proceedings of the National Academy of Sciences 96:14652-14657.

Böhlenius H., Huang T., Charbonnel-Campaa L., Brunner A.M., Jansson S., Strauss S.H., Nilsson O. (2006) CO/FT regulatory module controls timing of flowering and seasonal growth cessation in trees. Science 312:1040-1043.

Botto J.F., Sanchez R.A., Whitelam G.C., Casal J.J. (1996) Phytochrome A mediates the promotion of seed germination by very low fluences of light and canopy shade light in Arabidopsis. Plant physiology 110:439-444.

Briggs W.R., Christie J.M. (2002) Phototropins 1 and 2: versatile plant blue-light receptors. Trends in plant science 7:204-210.

Briggs W.R., Huala E. (1999) Blue-light photoreceptors in higher plants. Annual review of cell and developmental biology 15:33-62.

Bünning E., Moser I. (1973) Light-induced phase shifts of circadian leaf movements of Phaseolus: comparison with the effects of potassium and of ethyl alcohol. Proceedings of the National Academy of Sciences 70:3387-3389.

Bünning E., Zimmer R. (1962) Zur Deutung der Phasenverschiebungen und „transients" nach exogener Störung endogener Rhythmen. Planta 59:1-14.

Casal J.J., Yanovsky M.J. (2005) Regulation of gene expression by light. International Journal of Developmental Biology 49:501-511.

Cashmore A.R., Jarillo J.A., Wu Y.-J., Liu D. (1999) Cryptochromes: blue light receptors for plants and animals. Science 284:760-765.

Celaya R.B., Liscum E. (2005) Phototropins and Associated Signaling: Providing the Power of Movement in Higher Plants. Photochemistry and photobiology 81:73-80.

Ceriani M.F., Darlington T.K., Staknis D., Más P., Petti A.A., Weitz C.J., Kay S.A. (1999) Light-dependent sequestration of TIMELESS by CRYPTOCHROME. Science 285:553-556.

Chalker‐Scott L. (1999) Environmental significance of anthocyanins in plant stress responses. Photochemistry and photobiology 70:1-9.

Christie J.M. (2007) Phototropin blue-light receptors. Annual Review of Plant Biology 58:21-45.

Christie J.M., Briggs W.R. (2001) Blue light sensing in higher plants. Journal of Biological Chemistry 276:11457-11460.

Christie J.M., Reymond P., Powell G.K., Bernasconi P., Raibekas A.A., Liscum E., Briggs W.R. (1998) Arabidopsis NPH1: a flavoprotein with the properties of a photoreceptor for phototropism. Science 282:1698-1701.

Clack T., Mathews S., Sharrock R.A. (1994) The phytochrome apoprotein family inArabidopsis is encoded by five genes: the sequences and expression ofPHYD andPHYE. Plant molecular biology 25:413-427.

Collier D.E. (1997) Changes in respiration, protein and carbohydrates of tulip tepals and *Alstroemeria* petals during development. Journal of plant physiology 150:446-451.

Covington M.F., Panda S., Liu X.L., Strayer C.A., Wagner D.R., Kay S.A. (2001) ELF3 modulates resetting of the circadian clock in Arabidopsis. The Plant Cell 13:1305-1316.

Crosthwaite S.K., Dunlap J.C., Loros J.J. (1997) Neurospora wc-1 and wc-2: transcription, photoresponses, and the origins of circadian rhythmicity. Science 276:763-769.

Crosthwaite S.K., Loros J.J., Dunlap J.C. (1995) Light-induced resetting of a circadian clock is mediated by a rapid increase in frequency transcript. Cell 81:1003-1012.

Cushman L.C., Pemberton H.B., Kelly J.W. (1994) Cultivar, flower stage, silver thiosulfate, and BA interactions affect performance of potted miniature roses. HortScience 29:805-808.

de Mairan J. (1729) Observation botanique. Histoire de l'Academie Royale des Sciences:35-56.

Dehesh K., Franci C., Parks B.M., Seeley K.A., Short T.W., Tepperman J.M., Quail P.H. (1993) Arabidopsis HY8 locus encodes phytochrome A. The Plant Cell 5:1081-1088.

Deitzer G.F., Hayes R., Jabben M. (1979) Kinetics and time dependence of the effect of far red light on the photoperiodic induction of flowering in Wintex barley. Plant physiology 64:1015-1021.

Demarsy E., Fankhauser C. (2009) Higher plants use LOV to perceive blue light. Current opinion in plant biology 12:69-74.

Densmore R. (1997) Effect of day length on germination of seeds collected in Alaska. American Journal of Botany 84:274-274.

Devlin P.F., Kay S.A. (2000) Cryptochromes are required for phytochrome signaling to the circadian clock but not for rhythmicity. The Plant Cell 12:2499-2509.

Devlin P.F., Kay S.A. (2001) Circadian photoperception. Annual Review of Physiology 63:677-694.

Devlin P.F., Patel S.R., Whitelam G.C. (1998) Phytochrome E influences internode elongation and flowering time in Arabidopsis. The Plant Cell 10:1479-1487.

Devlin P.F., Robson P.R., Patel S.R., Goosey L., Sharrock R.A., Whitelam G.C. (1999) Phytochrome D acts in the shade-avoidance syndrome in Arabidopsis by controlling elongation growth and flowering time. Plant physiology 119:909-916.

Downs R. (1956) Photoreversibility of Flower Initiation. Plant physiology 31:279.

Dowson‐Day M.J., Millar A.J. (1999) Circadian dysfunction causes aberrant hypocotyl elongation patterns in Arabidopsis. The Plant Journal 17:63-71.

Doyle M.R., Davis S.J., Bastow R.M., McWatters H.G., Kozma-Bognár L., Nagy F., Millar A.J., Amasino R.M. (2002) The ELF4 gene controls circadian rhythms and flowering time in Arabidopsis thaliana. Nature 419:74-77.

Duhamel DuMonceau H. (1759) La Physique des Arbres. (Paris: H.L.Guerin and L.F. Delatour）.

Dunlap J.C. (1999) Molecular bases for circadian clocks. Cell 96:271-290.

Dunlap J.C., Loros J.J., DeCoursey P.J. (2004) Chronobiology: biological timekeeping Sinauer Associates.

Emery P., Stanewsky R., Hall J.C., Rosbash M. (2000) Drosophila cryptochromes: a unique circadian-rhythm photoreceptor. Nature 404:456-457.

Engelmann W., Johnsson A. (1998) Rhythms in organ movement. Biological rhythms and photoperiodism in plants:35-50.

Evans L. (1976) Inflorescence initiation in Lolium temulentum L. XIV. The role of phytochrome in long day induction. Functional Plant Biology 3:207-217.

Evans L., Borthwick H., Hendricks S. (1965) Inflorescence initiation in Lolium temulentum L. VII. The spectral dependence of induction. Australian Journal of Biological Sciences 18:745-762.

Ewusie J.Y., Quaye E. (1977) Diurnal periodicity in some common flowers. New Phytologist 78:479-485.

Farré E.M., Harmer S.L., Harmon F.G., Yanovsky M.J., Kay S.A. (2005) Overlapping and Distinct Roles of PRR7 and PRR9 in the Arabidopsis Circadian Clock. Current Biology 15:47-54.

Fernandez S.R., Wagner E. (1994) A New Method of Measurement and Analysis of the Stem Extension Growth Rate to Demonstrate Complete Synchronisation of *Chenopodium rubrum* Plants by Environmental Conditions. Journal of plant physiology 144:362-369.

Finlayson S.A., Lee I.-J., Morgan P.W. (1998) Phytochrome B and the regulation of circadian ethylene production in sorghum. Plant physiology 116:17-25.

Finlayson S.A., Lee I.-J., Mullet J.E., Morgan P.W. (1999) The mechanism of rhythmic ethylene production in sorghum. The role of phytochrome B and simulated shading. Plant physiology 119:1083-1090.

Foster K.R., Morgan P.W. (1995) Genetic Regulation of Development in Sorghum bicolor (IX. The ma3R Allele Disrupts Diurnal Control of Gibberellin Biosynthesis). Plant physiology 108:337-343.

Friend D. (1968a) Photoperiodic Responses of Brassica campestris cv. Ceres. Physiologia plantarum 21:990-1002.

Friend D. (1968b) Spectral Requirements for Flower Initiation in Two Longday Plants, Rape (Brassica campestris cv. Ceres), and Spring Wheat (Triticum× aestivum). Physiologia plantarum 21:1185-1195.

Fujiwara S., Ishida N., Tsuzuki M. (1996) Circadian expression of the carbonic anhydrase gene, Cah1, in Chlamydomonas reinhardtii. Plant molecular biology 32:745-749.

Giliberto L., Perrotta G., Pallara P., Weller J.L., Fraser P.D., Bramley P.M., Fiore A., Tavazza M., Giuliano G. (2005) Manipulation of the blue light photoreceptor cryptochrome 2 in tomato affects vegetative development, flowering time, and fruit antioxidant content. Plant physiology 137:199-208.

Gómez L.A., Simón E. (1995) Circadian rhythm of Robinia pseudoacacia leaflet movements: role of calcium and phytochrome. Photochemistry and photobiology 61:210-215.

Gorton H.L., Williams W.E., Assmann S.M. (1993) Circadian rhythms in stomatal responsiveness to red and blue light. Plant physiology 103:399-406.

Gorton H.L., Williams W.E., Binns M.E., Gemmell C.N., Leheny E.A., Shepherd A.C. (1989) Circadian stomatal rhythms in epidermal peels from Vicia faba. Plant Physiology 90:1329-1334.

Goto N., Kumagai T., Koornneef M. (1991) Flowering responses to light‐breaks in photomorphogenic mutants of Arabidopsis thaliana, a long‐day plant. Physiologia plantarum 83:209-215.

Griesel W.O. (1954) Retardation of Maturation in Magnolia Flowers by Maleic Hydrazide. Science (New York, NY) 119:843-845.

Halket A. (1931) The flowers of Silene saxifraga, L.; an inquiry into the cause of their day closure and the mechanism concerned in effecting their periodic movements. Annals of Botany:15-36.

Halliday K.J., Fankhauser C. (2003) Phytochrome‐hormonal signalling networks. New Phytologist 157:449-463.

Hamazato F., Shinomura T., Hanzawa H., Chory J., Furuya M. (1997) Fluence and wavelength requirements for Arabidopsis CAB gene induction by different phytochromes. Plant physiology 115:1533-1540.

Hammond J.B. (1982) Changes in amylase activity during rose bud opening. Scientia Horticulturae 16:283-289.

Harbaugh B.K. (2006) Lisianthus, Flower Breeding and Genetics, Springer. pp. 644-663.

Harmer S.L., Hogenesch J.B., Straume M., Chang H.-S., Han B., Zhu T., Wang X., Kreps J.A., Kay S.A. (2000) Orchestrated transcription of key pathways in Arabidopsis by the circadian clock. Science 290:2110-2113.

Hennessey T.L., Field C.B. (1991) Circadian rhythms in photosynthesis oscillations in carbon assimilation and stomatal conductance under constant conditions. Plant Physiology 96:831-836.

Hicks K.A., Albertson T.M., Wagner D.R. (2001) EARLY FLOWERING3 encodes a novel protein that regulates circadian clock function and flowering in Arabidopsis. The Plant Cell 13:1281-1292.

Hicks K.A., Millar A.J., Carré I.A., Somers D.E., Straume M., Meeks-Wagner D.R., Kay S.A. (1996) Conditional circadian dysfunction of the Arabidopsis early-flowering 3 mutant. Science 274:790-792.

Higuchi Y., Sage-Ono K., Sasaki R., Ohtsuki N., Hoshino A., Iida S., Kamada H., Ono M. (2011) 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 52:638-650.

Hisamatsu T., Koshioka M., Nishijima T., Mander L. (1998) Identification of endogenous gibberellins and their role in rosetted seedlings of Eustoma grandiflorum. Journal of the Japanese Society for Horticultural Science 67:866-871.

Ho L., Nichols R. (1977) Translocation of 14C-sucrose in relation to changes in carbohydrate content in rose corollas cut at different stages of development. Annals of Botany 41:227-242.

Horibe T., Yamada K. (2014) Petals of Cut Rose Flower Show Diurnal Rhythmic Growth. Journal of the Japanese Society for Horticultural Science 83.

Horie K. (1961) The behavior of the petals in the fading of the flowers ofTradescantia reflexa. Protoplasma 53:377-386.

Huang T., Böhlenius H., Eriksson S., Parcy F., Nilsson O. (2005) The mRNA of the Arabidopsis gene FT moves from leaf to shoot apex and induces flowering. Science 309:1694-1696.

Ichimura K., Suto K. (1998) Environmental Factors Controlling Flower Opening and Closing in aPortulacaHybrid. Annals of Botany 82:67-70.

Ievinsh G., Kreicbergs O. (1992) Endogenous rhythmicity of ethylene production in growing intact cereal seedlings. Plant physiology 100:1389-1391.

Imaizumi T., Tran H.G., Swartz T.E., Briggs W.R., Kay S.A. (2003) FKF1 is essential for photoperiodic-specific light signalling in Arabidopsis. Nature 426:302-306.

Imhoff C., Lecharny A., Jacques R., Brulfert J. (1979) Two phytochrome ‐ dependent processes in *Anagallis arvensis* L.: flowering and stem elongation. Plant, Cell and Environment 2:67-72.

Ishiguro S., Kawai-Oda A., Ueda J., Nishida I., Okada K. (2001) The DEFECTIVE IN ANTHER DEHISCENCE1 gene encodes a novel phospholipase A1 catalyzing the initial step of jasmonic acid biosynthesis, which synchronizes pollen maturation, anther dehiscence, and flower opening in Arabidopsis. The Plant Cell 13:2191-2209.

Iwasaki H., Kondo T. (2000) The current state and problems of circadian clock studies in cyanobacteria. Plant and cell physiology 41:1013-1020.

Jarillo J.A., Capel J., Tang R.-H., Yang H.-Q., Alonso J.M., Ecker J.R., Cashmore A.R. (2001) An Arabidopsis circadian clock component interacts with both CRY1 and phyB. Nature 410:487-490.

Johnson C.H., Knight M.R., Kondo T., Masson P., Sedbrook J., Haley A., Trewavas A. (1995) Circadian oscillations of cytosolic and chloroplastic free calcium in plants. Science 269:1863-1865.

Johnson E., Bradley M., Harberd N.P., Whitelam G.C. (1994) Photoresponses of light-grown phyA mutants of Arabidopsis (phytochrome A is required for the perception of daylength extensions). Plant physiology 105:141-149.

Kagawa T., Sakai T., Suetsugu N., Oikawa K., Ishiguro S., Kato T., Tabata S., Okada K., Wada M. (2001) Arabidopsis NPL1: a phototropin homolog controlling the chloroplast high-light avoidance response. Science 291:2138-2141.

Kaihara S., Takimoto A. (1979) Environmental factors controlling the time of flower-opening in Pharbitis nil. Plant and Cell Physiology 20:1659-1666.

Kaihara S., Takimoto A. (1980) Studies on the light controlling the time of flower-opening in Pharbitis nil. Plant and Cell Physiology 21:21-26.

Kaihara S., Takimoto A. (1981) Effects of light and temperature on flower-opening of Pharbitis nil. Plant and Cell Physiology 22:215-221.

Kaihara S., Takimoto A. (1983) Effect of plant growth regulators on flower-opening of Pharbitis nil. Plant and Cell Physiology 24:309-316.

Kami C., Lorrain S., Hornitschek P., Fankhauser C. (2010) Chapter two-light-regulated plant growth and development. Current topics in developmental biology 91:29-66.

Karvé A., Engelmann W., Schoser G. (1961) Initiation of rhythmical petal movements inKalanchoe blossfeldiana by transfer from continuous darkness to continuous light or vice versa. Planta 56:700-711.

Kasahara M., Torii M., Fujita A., Tainaka K. (2010) FMN binding and photochemical properties of plant putative photoreceptors containing two LOV domains, LOV/LOV proteins. Journal of Biological Chemistry 285:34765-34772.

Kawabata S., Nii K., Yokoo M. (2011) Three-dimensional formation of corolla shapes in relation to the developmental distortion of petals in *Eustoma grandiflorum*. Scientia Horticulturae 132:66-70.

Kay S.A., Keith B., Shinozaki K., Chye M.-L., Chua N.-H. (1989) The rice phytochrome gene: structure, autoregulated expression, and binding of GT-1 to a conserved site in the 5'upstream region. The Plant Cell 1:351-360.

Kendrick R., Kerckhoffs L., Tuinen A.v., Koornneef M. (1997) Photomorphogenic mutants of tomato. Plant, Cell and Environment 20:746-751.

Kendrick R.E., Kronenberg G. (1994) Photomorphogenesis in plants Springer.

Kim H.Y., Coté G.G., Crain R.C. (1993) Potassium channels in Samanea saman protoplasts controlled by phytochrome and the biological clock. Science 260:960-962.

Kim W.-Y., Fujiwara S., Suh S.-S., Kim J., Kim Y., Han L., David K., Putterill J., Nam H.G., Somers D.E. (2007) ZEITLUPE is a circadian photoreceptor stabilized by GIGANTEA in blue light. Nature 449:356-360.

Kircher S., Kozma-Bognar L., Kim L., Adam E., Harter K., Schäfer E., Nagy F. (1999) Light quality–dependent nuclear import of the plant photoreceptors phytochrome A and B. The Plant Cell 11:1445-1456.

Kolosova N., Gorenstein N., Kish C.M., Dudareva N. (2001) Regulation of circadian methyl benzoate emission in diurnally and nocturnally emitting plants. The Plant Cell Online 13:2333-2347.

Koning R.E. (1986) The role of ethylene in corolla unfolding in Ipomoea nil (Convolvulaceae). American Journal of Botany:152-155.

Koornneef M., Kendrick R.E. (1994) Photomorphogenic mutants of higher plants, Photomorphogenesis in plants, Springer. pp. 601-628.

Kunio Yamada, Rei Takahashi, Chiharu Fujitani, Keiko Mishima, Masato Yoshida, Daryl C. Joyce, Yamaki S. (2009) Cell wall extensibility and effect of cell-wall-loosening proteins during rose flower opening. Journal of the Japanese Society for Horticultural Science 78:242-251.

Lane H., Cathey H., Evans L. (1965) The dependence of flowering in several long-day plants on the spectral composition of light extending the photoperiod. American Journal of Botany:1006-1014.

Lecharny A., Wagner E. (1984) Stem extension rate in light ‐ grown plants. Evidence for an endogenous circadian rhythm in Chenopodium rubrum. Physiologia plantarum 60:437-443.

Leckie C., McAinsh M.R., Montgomery L., Priestley A., Staxen I., Webb A., Hetherington A.M. (1998) Second messengers in guard cells. Journal of experimental botany 49:339-349.

Lin C., Robertson D.E., Ahmad M., Raibekas A.A., Jorns M.S., Dutton P.L., Cashmore A.R. (1995) Association of flavin adenine dinucleotide with the Arabidopsis blue light receptor CRY1. Science 269:968-970.

Lin C., Shalitin D. (2003) Cryptochrome structure and signal transduction. Annual Review of Plant Biology 54:469-496.

Lin C., Yang H., Guo H., Mockler T., Chen J., Cashmore A.R. (1998) Enhancement of blue-light sensitivity of Arabidopsis seedlings by a blue light receptor cryptochrome 2. Proceedings of the National Academy of Sciences 95:2686-2690.

Liu X.L., Covington M.F., Fankhauser C., Chory J., Wagner D.R. (2001) ELF3 encodes a circadian clock–regulated nuclear protein that functions in an Arabidopsis PHYB signal transduction pathway. The Plant Cell 13:1293-1304.

Liu Y., Merrow M., Loros J.J., Dunlap J.C. (1998) How temperature changes reset a circadian oscillator. Science 281:825-829.

Liu Z., Taub C.C., McClung C.R. (1996) Identification of an Arabidopsis thaliana ribulose-1, 5-bisphosphate carboxylase/oxygenase activase (RCA) minimal promoter regulated by light and the circadian clock. Plant Physiology 112:43-51.

Love J., Dodd A.N., Webb A.A. (2004) Circadian and diurnal calcium oscillations encode photoperiodic information in Arabidopsis. The Plant Cell Online 16:956-966.

Lu Y., Gehan J.P., Sharkey T.D. (2005) Daylength and circadian effects on starch degradation and maltose metabolism. Plant physiology 138:2280-2291.

Lumsden P.J., Millar A.J. (1998) Biological rhythms and photoperiodism in plants. Bios Scientific Publishers Oxford.

Machackova I., Chauvaux N., Dewitte W., van Onckelen H. (1997) Diurnal fluctuations in ethylene formation in Chenopodium rubrum. Plant physiology 113:981-985.

Martínez-García J.F., Virgós-Soler A., Prat S. (2002) Control of photoperiod-regulated tuberization in potato by the Arabidopsis flowering-time gene CONSTANS. Proceedings of the National Academy of Sciences 99:15211-15216.

Más P., Kim W.-Y., Somers D.E., Kay S.A. (2003) Targeted degradation of TOC1 by ZTL modulates circadian function in Arabidopsis thaliana. Nature 426:567-570.

Mas P., Yanovsky M.J. (2009) Time for circadian rhythms: plants get synchronized. Current opinion in plant biology 12:574-579.

Matsushika A., Makino S., Kojima M., Mizuno T. (2000) Circadian waves of expression of the APRR1/TOC1 family of pseudo-response regulators in Arabidopsis thaliana: insight into the plant circadian clock. Plant and Cell Physiology 41:1002-1012.

McClung C.R. (2001) Circadian rhythms in plants. Annual review of plant biology 52:139-162.

McClung C.R. (2006) Plant circadian rhythms. The Plant Cell 18:792-803.

McWatters H.G., Bastow R.M., Hall A., Millar A.J. (2000) The ELF3 zeitnehmer regulates light signalling to the circadian clock. Nature 408:716-720.

Millar A.J. (1997) Circadian rhythms: PASsing time. Current Biology 7:R474-R476.

Millar A.J. (1999) Biological clocks in Arabidopsis thaliana. New phytologist 141:175-197.

Millar A.J., Kay S.A. (1991) Circadian control of cab gene transcription and mRNA accumulation in Arabidopsis. The Plant Cell Online 3:541-550.

Millar A.J., Kay S.A. (1996) Integration of circadian and phototransduction pathways in the network controlling CAB gene transcription in Arabidopsis. Proceedings of the National Academy of Sciences 93:15491-15496.

Millar A.J., Straume M., Chory J., Chua N.-H., Kay S.A. (1995) The regulation of circadian period by phototransduction pathways in Arabidopsis. Science 267:1163-1166.

Miyamoto Y., Sancar A. (1998) Vitamin B2-based blue-light photoreceptors in the retinohypothalamic tract as the photoactive pigments for setting the circadian clock in mammals. Proceedings of the National Academy of Sciences 95:6097-6102.

Morgan P., Finlayson S., Lee I., Childs K., He C.-J., Creelman R., Drew M., Mullet J. (1997) Regulation of circadianly rhythmic ethylene production by phytochrome B in sorghum, Biology and Biotechnology of the Plant Hormone Ethylene, Springer. pp. 105-111.

Moshelion M., Becker D., Czempinski K., Mueller-Roeber B., Attali B., Hedrich R., Moran N. (2002) Diurnal and circadian regulation of putative potassium channels in a leaf moving organ. Plant Physiology 128:634-642.

Moysset L., Gómez L.A., Simón E. (1994) Effects of lanthanum on rhythmic and nyctinastic leaflet movements in Albizzia lophantha. Journal of Experimental Botany 45:85-93.

Nagatani A., Reed J.W., Chory J. (1993) Isolation and initial characterization of Arabidopsis mutants that are deficient in phytochrome A. Plant physiology 102:269-277.

Naidoo N., Song W., Hunter-Ensor M., Sehgal A. (1999) A role for the proteasome in the light response of the timeless clock protein. Science 285:1737-1741.

Nakamichi N., Kiba T., Henriques R., Mizuno T., Chua N.-H., Sakakibara H. (2010) PSEUDO-RESPONSE REGULATORS 9, 7, and 5 are transcriptional repressors in the Arabidopsis circadian clock. The Plant Cell 22:594-605.

Nakamichi N., Kita M., Ito S., Yamashino T., Mizuno T. (2005) PSEUDO-RESPONSE REGULATORS, PRR9, PRR7 and PRR5, together play essential roles close to the circadian clock of Arabidopsis thaliana. Plant and cell physiology 46:686-698.

Nakanishi F., Nakazawa M., Katayama N. (2005) Opening and closing of Oxalis leaves in response to light stimuli. Journal of Biological Education 39:87-91.

Neff M.M., Chory J. (1998) Genetic interactions between phytochrome A, phytochrome B, and cryptochrome 1 during Arabidopsis development. Plant physiology 118:27-35.

Nelson D.C., Lasswell J., Rogg L.E., Cohen M.A., Bartel B. (2000) *FKF1*, a Clock-Controlled Gene that Regulates the Transition to Flowering in *Arabidopsis*. Cell 101:331-340.

Nemhauser J., Chory J. (2002) Photomorphogenesis. Arabidopsis Book 1:e0054.

Ni M., Tepperman J.M., Quail P.H. (1999) Binding of phytochrome B to its nuclear signalling partner PIF3 is reversibly induced by light. Nature 400:781-784.

Niinuma K., Nakagawa M., Calvino M., Mizoguchi T. (2007) Dance of plants with circadian clock. Plant biotechnology 24:87-97.

Niinuma K., Someya N., Kimura M., Yamaguchi I., Hamamoto H. (2005) Circadian rhythm of circumnutation in inflorescence stems of Arabidopsis. Plant and Cell Physiology 46:1423-1427.

Niramo H.G. (1998) Circadian regulation of a plant protein kinase. Chronobiol Int 15:109-118.

Niu Y., Yang Y., Zhang Z.-Q., Li Z.-M., Sun H. (2011) Floral closure induced by pollination in gynodioecious Cyananthus delavayi (Campanulaceae): effects of

pollen load and type, floral morph and fitness consequences. Annals of Botany:mcr224.

O'Donoghue E.M., Somerfield S.D., Heyes J.A. (2002a) Organization of cell walls in Sandersonia aurantiaca floral tissue. Journal of Experimental Botany 53:513-523.

O'Donoghue E.M., Somerfield S.D., Heyes J.A. (2002b) Vase solutions containing sucrose result in changes to cell walls of sandersonia (*Sandersonia aurantiaca*) flowers. Postharvest Biology and Technology 26:285-294.

Ochiai M., Matsumoto S., Maesaka M. (2013) Expression of mRNAs and Proteins Associated with Cell-wall-loosening during Eustoma Flower Opening. Journal of the Japanese Society for Horticultural Science 82:154-160.

Ogura Y., Komatsu A., Zikihara K., Nanjo T., Tokutomi S., Wada M., Kiyosue T. (2008) Blue light diminishes interaction of PAS/LOV proteins, putative blue light receptors in Arabidopsis thaliana, with their interacting partners. Journal of plant research 121:97-105.

Ohkawa K., Kano A., Kanematsu K., Korenaga M. (1991) Effects of air temperature and time on rosette formation in seedlings of *Eustoma grandiflorum* (Raf.) Shinn. Scientia Horticulturae 48:171-176.

Overland L. (1960) Endogenous rhythm in opening and odor of flowers of Cestrum nocturnum. American Journal of Botany:378-382.

Parks B.M., Spalding E.P. (1999) Sequential and coordinated action of phytochromes A and B during Arabidopsis stem growth revealed by kinetic analysis. Proceedings of the National Academy of Sciences 96:14142-14146.

Pesti J. (1976) Daily fluctuations in the sugar content of nectar and periodicity of secretion in the Compositae. Acta Agron Budap 25:5-17.

Pfeffer W. (1873) Physiologische Untersuchungen (Leipzig, Germany: W. Engelmann).

Phillips Jr H., Kende H. (1980) Structural changes in flowers ofIpomoea tricolor during flower opening and closing. Protoplasma 102:199-215.

Pittendrigh C.S. (1954) On temperature independence in the clock system controlling emergence time in Drosophila. Proceedings of the National Academy of Sciences of the United States of America 40:1018.

Pokhilko A., Fernández A.P., Edwards K.D., Southern M.M., Halliday K.J., Millar A.J. (2012) The clock gene circuit in Arabidopsis includes a repressilator with additional feedback loops. Molecular systems biology 8:574.

Primack R.B. (1985) Patterns of flowering phenology in communities, populations, individuals, and single flowers, The population structure of vegetation, Springer. pp. 571-593.

Quail P.H., Boylan M.T., Parks B.M., Short T.W., Xu Y., Wagner D. (1995) Phytochromes: photosensory perception and signal transduction. Science 268:675-680.

Ramos A., Pérez-Solís E., Ibáñez C., Casado R., Collada C., Gómez L., Aragoncillo C., Allona I. (2005) Winter disruption of the circadian clock in chestnut. Proceedings of the National Academy of Sciences of the United States of America 102:7037-7042.

Reed J.W., Nagatani A., Elich T.D., Fagan M., Chory J. (1994) Phytochrome A and phytochrome B have overlapping but distinct functions in Arabidopsis development. Plant physiology 104:1139-1149.

Reed J.W., Nagpal P., Poole D.S., Furuya M., Chory J. (1993) Mutations in the gene for the red/far-red light receptor phytochrome B alter cell elongation and physiological responses throughout Arabidopsis development. The Plant Cell 5:147-157.

Reid H., Moore P., Hamner K. (1967) Control of Flowering of Xanthium pensylvanicum by Red and Far-red Light. Plant physiology 42:532-540.

Reid M., Evans R., Dodge L., Mor Y. (1989) Ethylene and silver thiosulfate influence opening of cut rose flowers. Journal of the American Society for Horticultural Science 114:436-440.

Ritz D., Kluge M. (1987) Circadian Rhythmicity of CAM in Continuous Light: Coincidences between Gas Exchange Parameters, $^{14}CO_2$ Fixation Patterns and PEP-carboxylase Properties. Journal of plant physiology 131:285-296.

Robertson McClung C. (2000) Circadian rhythms in plants: a millennial view. Physiologia Plantarum 109:359-371.

Rockwell N.C., Su Y.-S., Lagarias J.C. (2006) Phytochome structure and signaling mechanisms. Annual Review of Plant Biology 57:837.

Runkle E.S., Heins R.D. (2001) Specific functions of red, far red, and blue light in flowering and stem extension of long-day plants. Journal of the American Society for Horticultural Science 126:275-282.

Sakai T., Kagawa T., Kasahara M., Swartz T.E., Christie J.M., Briggs W.R., Wada M., Okada K. (2001) Arabidopsis nph1 and npl1: blue light receptors that mediate both phototropism and chloroplast relocation. Proceedings of the National Academy of Sciences 98:6969-6974.

Salomé P.A., McClung C.R. (2005) PSEUDO-RESPONSE REGULATOR 7 and 9 are partially redundant genes essential for the temperature responsiveness of the Arabidopsis circadian clock. The Plant Cell 17:791-803.

Salter M.G., Franklin K.A., Whitelam G.C. (2003) Gating of the rapid shade-avoidance response by the circadian clock in plants. Nature 426:680-683.

Sanders D., Brownlee C., Harper J.F. (1999) Communicating with calcium. The Plant Cell Online 11:691-706.

Satter R.L., Gorton H.L., Vogelmann T.C. (1990) The pulvinus: motor organ for leaf movement American Society of Plant Physiologists.

Schaffer R., Landgraf J., Accerbi M., Simon V., Larson M., Wisman E. (2001) Microarray analysis of diurnal and circadian-regulated genes in Arabidopsis. The Plant Cell 13:113-123.

Schaffer R., Ramsay N., Samach A., Corden S., Putterill J., Carre I.A., Coupland G. (1998) The late elongated hypocotyl mutation of Arabidopsis disrupts circadian rhythms and the photoperiodic control of flowering. Cell 93:1219-29.

Schroeder J.I., Allen G.J., Hugouvieux V., Kwak J.M., Waner D. (2001) Guard cell signal transduction. Annual review of plant biology 52:627-658.

Schultz T.F., Kiyosue T., Yanovsky M., Wada M., Kay S.A. (2001) A role for LKP2 in the circadian clock of Arabidopsis. The Plant Cell 13:2659-2670.

Schulze E., Turner N., Gollan T., Shackel K. (1987) Stomatal responses to air humidity and to soil drought. Stomatal function 804713472:311-321.

Searle I., Coupland G. (2004) Induction of flowering by seasonal changes in photoperiod. The EMBO Journal 23:1217-1222.

Serek M., Jones R.B., Reid M.S. (1994) Role of ethylene in opening and senescence of Gladiolus sp. flowers. Journal of the American Society for Horticultural Science 119:1014-1019.

Sharkey T., Ogawa T., Zeiger E., Farquhar G., Cowan I. (1987) Stomatal responses to light. Stomatal function:195-208.

Sharrock R.A., Quail P.H. (1989) Novel phytochrome sequences in Arabidopsis thaliana: structure, evolution, and differential expression of a plant regulatory photoreceptor family. Genes and Development 3:1745-1757.

Shimizu-Yumoto H., Ichimura K. (2010) Combination pulse treatment of 1-naphthaleneacetic acid and aminoethoxyvinylglycine greatly improves postharvest life in cut *Eustoma* flowers. Postharvest Biology and Technology 56:104-107.

Shimizu-Yumoto H., Ichimura K. (2012) Effects of ethylene, pollination, and ethylene inhibitor treatments on flower senescence of gentians. Postharvest Biology and Technology 63:111-115.

Shinners L.H. (1957) Synopsis of the genus Eustoma (Gentianaceae). The Southwestern Naturalist:38-43.

Shinomura T., Nagatani A., Hanzawa H., Kubota M., Watanabe M., Furuya M. (1996) Action spectra for phytochrome A-and B-specific photoinduction of seed germination in Arabidopsis thaliana. Proceedings of the National Academy of Sciences 93:8129-8133.

Shinomura T., Uchida K., Furuya M. (2000) Elementary processes of photoperception by phytochrome A for high-irradiance response of hypocotyl elongation in Arabidopsis. Plant physiology 122:147-156.

Sigmond H. (1929) Über das Aufblühen von *Hedera helix* L. und Beeinflussung dieses Vorganges durch das Licht. Beihefte des Botanisches Zentralblatt 46:68-92.

Sigmond H. (1930) Die Entfaltung Bluütenknospe zweier *Oenothera*-Arten. Teil II. Beihefte des Botanisches Zentralblatt 47:69-138.

Smith H., Whitelam G. (1990) Phytochrome, a family of photoreceptors with multiple physiological roles. Plant, Cell & Environment 13:695-707.

Smith H., Xu Y., Quail P.H. (1997) Antagonistic but complementary actions of phytochromes A and B allow optimum seedling de-etiolation. Plant physiology 114:637-641.

Somers D.E. (1999) The physiology and molecular bases of the plant circadian clock. Plant Physiology 121:9-20.

Somers D.E., Devlin P.F., Kay S.A. (1998a) Phytochromes and cryptochromes in the entrainment of the Arabidopsis circadian clock. Science 282:1488-1490.

Somers D.E., Fujiwara S. (2009) Thinking outside the F-box: novel ligands for novel receptors. Trends in plant science 14:206-213.

Somers D.E., Schultz T.F., Milnamow M., Kay S.A. (2000) ZEITLUPE Encodes a Novel Clock-Associated PAS Protein from Arabidopsis. Cell 101:319-329.

Somers D.E., Webb A., Pearson M., Kay S.A. (1998b) The short-period mutant, toc1-1, alters circadian clock regulation of multiple outputs throughout development in Arabidopsis thaliana. Development 125:485-494.

Staiger D., Zecca L., Kirk D.A.W., Apel K., Eckstein L. (2003) The circadian clock regulated RNA‐binding protein AtGRP7 autoregulates its expression by influencing alternative splicing of its own pre‐mRNA. The Plant Journal 33:361-371.

Strayer C., Oyama T., Schultz T.F., Raman R., Somers D.E., Mas P., Panda S., Kreps J.A., Kay S.A. (2000) Cloning of the Arabidopsis clock gene TOC1, an autoregulatory response regulator homolog. Science 289:768-71.

Symons G.M., Reid J.B. (2003) Interactions between light and plant hormones during de-etiolation. Journal of Plant Growth Regulation 22:3-14.

Tanaka O., Murakami H., Wada H., Tanaka Y., Naka Y. (1989) Flower opening and closing of Oxalis martiana. The botanical magazine= Shokubutsu-gaku-zasshi 102:245-253.

Taylor B.L., Zhulin I.B. (1999) PAS domains: internal sensors of oxygen, redox potential, and light. Microbiology and Molecular Biology Reviews 63:479-506.

Thomas B., Vince-Prue D. (1996) Photoperiodism in plants Academic Press.

Tischkau S.A., Gallman E.A., Buchanan G.F., Gillette M.U. (2000) Differential cAMP gating of glutamatergic signaling regulates long-term state changes in the suprachiasmatic circadian clock. The Journal of Neuroscience 20:7830-7837.

Tjosvold S.A., Wu M.-J., Reid M.S. (1994) Reduction of postproduction quality loss in potted miniature roses. HortScience 29:293-294.

Tukey Jr H., Ketellapper H. (1963) Length of the light-dark cycle and plant growth. American Journal of Botany:110-115.

Van Doorn W.G. (1997) Effects of pollination on floral attraction and longevity. Journal of Experimental Botany 48:1615-1622.

van Doorn W.G., van Meeteren U. (2003) Flower opening and closure: a review. Journal of Experimental Botany 54:1801-1812.

Van Meeteren U., Van Gelder H., Van De Peppel A. (1995) Aspects of carbohydrate balance during floret opening in Freesia, VI International Symposium on Postharvest Physiology of Ornamental Plants 405. pp. 117-122.

Van Tuinen A., Kerckhoffs L., Nagatani A., Kendrick R., Koornneef M. (1995) Far-red light-insensitive, phytochrome A-deficient mutants of tomato. Molecular and General Genetics MGG 246:133-141.

Vince-Prue D. (1983) Photomorphogenesis and flowering, Photomorphogenesis, Springer. pp. 457-490.

Vince-Prue D. (1994) The duration of light and photoperiodic responses, Photomorphogenesis in plants, Springer. pp. 447-490.

Wagner D., Tepperman J.M., Quail P.H. (1991) Overexpression of phytochrome B induces a short hypocotyl phenotype in transgenic Arabidopsis. The Plant Cell 3:1275-1288.

Wang Z.-Y., Tobin E.M. (1998) Constitutive Expression of the CIRCADIAN CLOCK ASSOCIATED 1 (CCA1) Gene Disrupts Circadian Rhythms and Suppresses Its Own Expression. Cell 93:1207-1217.

Webb A.A. (2003) The physiology of circadian rhythms in plants. New Phytologist 160:281-303.

Whitelam G., Devlin P. (1997) Roles of different phytochromes in Arabidopsis photomorphogenesis. Plant, Cell and Environment 20:752-758.

Whitelam G.C. (1998) Phytochromes and photomorphogenesis in Arabidopsis. Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences 353:1445-1453.

Whitelam G.C., Johnson E., Peng J., Carol P., Anderson M.L., Cowl J.S., Harberd N.P. (1993) Phytochrome A null mutants of Arabidopsis display a wild-type phenotype in white light. The Plant Cell 5:757-768.

Wiedersheim W. (1904) Studien über photonastische und thermonastische Bewegungen.

Wijnen H., Young M.W. (2006) Interplay of circadian clocks and metabolic rhythms. Annual Review of Genetics 40:409-448.

Wood N.T., Haley A., Viry-Moussaıd M., Johnson C.H., van der Luit A.H., Trewavas A.J. (2001) The calcium rhythms of different cell types oscillate with different circadian phases. Plant Physiology 125:787-796.

Yakir E., Hilman D., Harir Y., Green R.M. (2007) Regulation of output from the plant circadian clock. Febs Journal 274:335-345.

Yamamoto K., Komatsu Y., Yokoo Y., Furukawa T. (1994) Delaying flower opening of cut roses by cis-propenylphosphonic acid. Journal of the Japanese Society for Horticultural Science 63:159-166.

Yamane K., Kawabata S., Sakiyama R. (1991) Changes in water relations, carbohydrate contents, and acid invertase activity associated with perianth elongation during anthesis of cut gladiolus flowers. Journal of the Japanese Society for Horticultural Science 60:421-424.

Yanovsky M.J., Kay S.A. (2001) Signaling networks in the plant circadian system. Current opinion in plant biology 4:429-435.

Yanovsky M.J., Mazzella M.A., Casal J.J. (2000) A quadruple photoreceptor mutant still keeps track of time. Current Biology 10:1013-1015.

Yanovsky M.J., Mazzella M.A., Whitelam G.C., Casal J.J. (2001) Resetting of the circadian clock by phytochromes and cryptochromes in Arabidopsis. Journal of biological rhythms 16:523-530.

Young M.W. (1998) The molecular control of circadian behavioral rhythms and their entrainment in Drosophila. Annual review of biochemistry 67:135-152.

Zheng C.C., Porat R., Lu P., O'Neill S.D. (1998) PNZIP is a novel mesophyll-specific cDNA that is regulated by phytochrome and a circadian rhythm and encodes a protein with a leucine zipper motif. Plant Physiology 116:27-35.

Zhong H.H., McClung C.R. (1996) The circadian clock gates expression of twoArabidopsis catalase genes to distinct and opposite circadian phases. Molecular and General Genetics MGG 251:196-203.

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