Petals of Cut Rose Flower Show Diurnal Rhythmic Growth

Takanori Horibe and Kunio Yamada*

College of Bioscience and Biotechnology, Chubu University, Kasugai 487-8501, Japan

Some flowers including rose open in a rhythmic fashion during specific times of the day. We used time-lapse cinematography to understand the mechanism of rhythmic opening and perception of light in cut rose flowers. Cut rose flowers exposed to a 12 h light/12 h dark photoperiod started opening shortly before the light period had begun and stopped during the light period even when their leaves were removed, indicating that petals and/or sepals perceive light and synchronize flower opening to photoperiods. This rhythmic opening could be seen in constant darkness even though the time of flower opening shifted to an earlier point in constant darkness compared with the 12 h photoperiod, but it was not observed in constant light. We also evaluated the effect of exposing cut flowers first to a 12 h photoperiod and then shifting them to an 18 h photoperiod. During the 12 h photoperiod, flower opening started shortly before the light period had begun and stopped during the light period, while in the 18 h photoperiod, it proceeded in the middle of the dark period. These results suggested that changes of light to darkness or vice versa were important signals for the start and maintenance of rhythmic flower opening. In addition, we found that even a petal removed from a rose flower showed rhythmic growth when exposed to a 12 h light/12 h dark photoperiod, showing that petals could perceive light and synchronize their growth to the photoperiod.

Key Words: circadian rhythm, cut rose, flower opening, petal growth, photoperiod.

Introduction

Rhythmic flower opening can be observed in many plant species, and an endogenous rhythm of opening is usually identified by placing in constant darkness and constant light. Some day-bloomer species and night-bloomer species have been shown to have an endogenous rhythm of flower opening and closure (van Doorn and van Meeteren, 2003). However, changing from light to darkness or vice versa is necessary for flower opening in several species. In the Asiatic hybrid lily (Lilium hybrid), flower opening proceeded irregularly if it was kept in continuous darkness, indicating the importance of changing from light to darkness for the timing of flower opening (Bielieski et al., 2000). Rose-flower opening is a process of irreversible petal growth and reflection in which existing cells expand and fresh and dry weights increase (Evans and Reid, 1986, 1988; Faragher et al., 1984). In hybrid tea rose, flower opening showed a diurnal rhythm under a 12 h photoperiod, but proceeded irregularly if it was kept in continuous darkness (Evans and Reid, 1986, 1988). This indicated the importance of changing from light to darkness for the timing of flower opening. Several studies have indicated that petals can perceive light and synchronise their growth to photoperiods. In Calendula arvensis, the opening and closing rhythm of flowers followed the light/dark cycle to which the flower had been exposed when the leaves were subjected to a different cycle from the flowers (van Doorn and van Meeteren, 2003). Furthermore, in Ipomoea nil, the petals were found to be the site of photoperception (Kaihara and Takimoto, 1980, 1981a, b). However, it is still unclear whether rose petals can perceive light and adjust their growth to photoperiods.

Elucidation of the mechanisms of flower opening would contribute to the commercial production of floricultural crops as well as general plant science. Until now, research has focused on senescence to improve the vase life of flowers (van Doorn and Woltering, 2008), leading to the invention of ethylene production/action inhibitors. However, few studies have focused on the perception of light and the effects of light on the opening of rose flowers. Some studies have shown that the light condition affects several aspects of plant physiology, including flower opening and volatile emission (Hendel-Rahmanim et al., 2007; Kaihara and Takimoto, 1980,
1981a, b). Understanding a cut rose flower’s response to light stimuli might lead to the development of a new method to improve its qualities. Therefore, in this research, we further investigated the rhythmic opening of rose flowers using a cut rose without leaves and a petal to clarify the effect of light stimuli on flower opening, and whether petals can perceive light and synchronize their growth to photoperiods by time-lapse cinematography.

**Materials and Methods**

**Plant materials**

Roses (*Rosa ‘FEbesa’*), known in Japan as ‘Pretty Woman’, were obtained from the farm of Nagoya University in Aichi Prefecture, Japan. The flowers were harvested at two stages: ST1, sepals were closed and petals were pigmented (younger than the commercial harvesting stage); ST2, sepals were separated completely from each other. Cut flowers were harvested around 10:00 a.m. and transported in a wet condition to our laboratory within 1 h. Sunrise was around 6:00 a.m. in Aichi Prefecture when we harvested the flowers.

**Cut flower treatment and time-lapse cinematography**

Soon after arrival at our laboratory, the stems, with the leaves removed, were re-cut in water to 11-cm length. All treatments were started around 12:00 p.m. They were kept in deionized water at 25°C, 55% relative humidity. All cut flowers (ST2) were exposed to one cycle of a 12 h photoperiod (220–240 μmol·m−2·s−1), and then kept under a 12 h photoperiod, in constant darkness, and in constant light (220–240 μmol·m−2·s−1). We also observed the effect of changing the photoperiod on rhythmic flower opening. Cut flowers were kept under an 18 h photoperiod after exposure to a 12 h photoperiod. Flower opening was recorded every 30 min by digital camera (Coolpix s1; Nikon Corporation, Tokyo, Japan) and changes of flower diameter at the outermost petals and inner petals (except for the outer 6 petals) were measured using ImageJ software (Fig. 1). During the dark period, we used the flash function of the camera to take pictures. We measured changes in the flower diameter of cut flowers ($L_n$) every 30 min to calculate the growth rate (GR), $GR = \frac{L_{n+1}}{L_n}$.

**Time-lapse cinematography of rose petal**

The cut flower (ST1) was kept in darkness for 24 h at 4°C soon after arrival at our laboratory. After that, a rose petal was picked from the cut flower after the outer 5 petals had been removed. Its base was held in purified water at 25°C, 55% relative humidity, and a 12 h photoperiod (220–240 μmol·m−2·s−1). Petal growth was recorded every 30 min by digital camera (Coolpix s1; Nikon Corporation) and changes of petal width (middle region) were measured using ImageJ software (Fig. 1). During the dark period, we used the flash function of the camera to take pictures. We measured changes in petal width ($W_n$) every 30 min to calculate the GR, $GR = \frac{W_{n+1}}{W_n}$.

**Results**

**Changes in flower diameter in each photoperiod**

Changes in the GR of the flower diameter are shown in Figure 2. They increased immediately during the first light period (Fig. 2, 0–12 h), and then showed a rhythmic pattern, peaking soon after the light period had started, indicating that the flower opened rapidly at that time. The last peak of the GR around 72 h after treatment was lower than the previous two peaks, probably because...
the flower had almost fully opened before that time. Cut flowers exposed to constant darkness showed a diurnal rhythm of flower opening, although the GR peaks around 72 h shifted to earlier time points than those of cut flowers exposed to 12 h photoperiods (Fig. 3). In addition, the GR peaks around 48 h and 72 h were lower than the peak around 24 h and the flower kept opening slowly between these peaks. In contrast, rhythmic flower opening was not observed in cut flowers exposed to constant light (Fig. 4). Rapid flower opening was observed after one cycle of a 12 h photoperiod, but then the flower kept opening gradually until it had fully opened.

Next, we evaluated the effect of the changing photoperiod from 12 h to 18 h (Fig. 5). We measured diameter in inner petals from 30 h after treatment, because it was hard to measure it until then. During the 12 h photoperiod, the GR of the flower diameter peaked soon after the light period had started, while in the 18 h photoperiod, it increased in the middle of the dark period and shortly after the light period had begun, although changes in flower diameter were small. This is because the flower had almost fully opened at that time, and changes in flower diameter were therefore small. In all experiments, the GR of the flower diameter tended to rise during the first light period (Figs. 2, 3, 4, and 5).

**Fig. 3.** GR of diameter of cut flowers exposed to constant darkness after one cycle of 12 h photoperiod. Periods of darkness and light are indicated by shaded and non-shaded areas, respectively. Each value is the mean±SE (n=4).

**Fig. 4.** GR of diameter of cut flowers exposed to constant light after one cycle of 12 h photoperiod. Periods of darkness and light are indicated by shaded and non-shaded areas, respectively. Each value is the mean±SE (n=4).

**Fig. 5.** GR of flower diameter in outermost petals (A) and inner petals (B) in cut flowers exposed to 12 h and 18 h photoperiods. Periods of darkness and light are indicated by shaded and non-shaded areas, respectively. Each value is the mean±SE (n=4).

**Fig. 6.** GR of the width of petals exposed to 12 h light/12 h dark photoperiod. Periods of darkness and light are indicated by shaded and non-shaded areas, respectively. Each value is the mean±SE (n=3).
Changes in the GR of the petal width are shown in Figure 6. It also increased shortly after treatment had started, and then it showed diurnal changes, peaking soon after the light period had begun, indicating that petals grow in a rhythmic fashion. The last peak of the GR was much lower than the previous peak.

Discussion

Rose flowers have been shown to open in a rhythmic fashion in some studies (Doi et al., 1999; Evans and Reid, 1986, 1988). Thus, to begin with, we investigated whether leaves are necessary for rhythmic opening of cut rose flowers. Cut flowers exposed to a 12 h photoperiod showed rhythmic flower opening, starting shortly before the light period and lasting a few hours, although all of its leaves were removed (Fig. 2). This indicates that petals, sepals, and/or stem can perceive light and synchronize flower opening to the photoperiod. Next, we tested whether this rhythmic flower opening is controlled by the circadian clock, as the activity of the circadian clock continues under constant dark and light conditions (Jones and Mansfield, 1975). Cut flowers held in constant dark condition showed rhythmic flower opening, although rhythmic flower opening was not as clear as that of cut flowers kept under 12 h light and 12 h dark cycles, as the flower held under constant darkness kept opening slowly between its peaks (Fig. 3). The periods and amplitude of circadian rhythm are known to change without zeitgeber, such as changes of light to darkness or vice versa (Jones and Mansfield, 1975). During constant darkness, stoppage of the light signal seemed to have caused changes in the rhythmic growth of flower opening. On the other hand, the diurnal rhythm of cut flower opening was abolished when exposed to constant light (Fig. 4). Cut flowers in constant light kept opening slowly until they had fully opened. These results suggest that the rhythmic opening of cut rose flowers is influenced by changes of light to darkness or vice versa, in addition to circadian factors. The mechanism of rhythmic flower opening has not been elucidated yet. Some chemical reactions which connect the circadian oscillator and flower opening might be halted under constant light conditions. It therefore seems that the opening of rose flowers needs external stimuli, a change of light to darkness or vice versa, to maintain its diurnal rhythm. Interestingly, some volatile compounds of rose flowers are emitted in a similar way. Emission of geranyl acetate oscillated under 12 h light/dark conditions, but not in constant light, indicating that cyclic light and darkness are necessary for diurnal volatile emission (Hendel-Rahmanin et al., 2007). Thus, it seems that changes of light to darkness or vice versa play a key role in diverse phenomena in plants. However, Evans and Reid (1986) reported that rhythmic opening of rose flowers was abolished by placing flowers in continuous light or darkness, while Doi et al. (1999) reported that it was observed when flowers were exposed to constant light and darkness. These differences might result from excision of leaves or differences between rose cultivars in the mechanism of rhythmic flower opening. We also evaluated the effect of the changing photoperiod from 12 h to 18 h (Fig. 5). When cut flowers were shifted from a 12 h to an 18 h photoperiod, flowers opened in the middle of the dark period. The finding that flowers opened in the middle of the 18 h dark period might indicate that the time of flower opening started several hours after changing from light to darkness. Moreover, when cut flowers were shifted from 12 h darkness to constant light, the diurnal rhythm of flower opening was abolished (Fig. 4). So it seems that changing from light to darkness and a certain period of darkness are necessary to maintain the diurnal rhythm of flower opening. Further research is warranted to understand how the time of flower opening is determined. These results also support the idea that changing from light to darkness or vice versa is an important signal for the rhythmic opening of rose cut flowers. In addition, the GR peak around 18 h was much higher than peaks observed in other experiments, such as cut flowers kept under 12 h photoperiods, constant darkness, and constant light (Figs. 2, 3, 4, and 5A). Sunrise was around 6:00 a.m. when we harvested cut flowers, and we started treatments at 12:00 a.m., which means that cut flowers had been kept under light conditions for about 6 h when treatments were started. In this experiment, cut flowers were exposed to light for 6 h in the first light period so that cut flowers had been kept under light for 12 h when the first dark period began to evaluate the effect of changing the photoperiod from 12 h to 18 h (Fig. 5A). On the other hand, in other experiments, cut flowers had been exposed to light for about 18 h when the first dark period began. Differences in these light periods during treatments and other environmental conditions such as light intensity and temperature before treatments might have affected the rhythmic opening of cut flowers and resulted in changes in GR peaks between treatments.

Moreover, we noticed that flowers opened shortly after the light period had begun in the 18 h photoperiod (Fig. 5) and during the first light periods in all experiments (Figs. 2, 3, 4, 5, and 6). This phenomenon might indicate that light itself acts as a positive signal in flower opening. However, there is a possibility that light increased petal temperature by being absorbed by pigments in petals, resulting in petal growth. This rise in temperature has positive effects on flower opening in some species. In Portulaca plants, for example, the rise in temperature resulted in rapid flower opening, although light intensified the response (Ichimura and Suto, 1998).

Cut flowers without leaves showed rhythmic flower opening, suggesting the possibility that rose petals could perceive light and adjust their growth to photoperiods. We found that rose petals show rhythmic growth starting shortly before the light period and lasting a few hours, when exposed to a 12 h photoperiod (Fig. 6). This result
indicates that petals are the site of photoperception and can synchronize their growth to the photoperiod. We used petals from ST1 cut flowers in this experiment, because petals from ST2 flowers were relatively larger than ST1 petals and showed little change in petal size, although they showed rhythmic growth (data not shown). In *Ipomoea nil*, red light promoted flower opening and its effect was reversed by subsequent exposure to far-red light (Kaihara and Takimoto, 1980, 1981a, b). Another report showed that a relatively slow phytochrome reaction is involved in the perception of the duration of light or darkness (Lumsden, 1991). Such a reaction might also be involved in light perception in roses; however, it is still not clear which wavelength is effective and which photoreceptor perceives light in rose petals. Such studies are now in progress in our laboratory. We used a flash to take pictures of flower opening during dark periods. Cut flowers without leaves kept under a 12 h photoperiod and not exposed to the flash also showed rhythmic flower opening, which proceeded rapidly soon after the light period had begun (data not shown). Thus, we think that the effect of a flash on rhythmic flower opening is not significant and that we can evaluate the effect of photoperiods even using a flash. However, some phytochrome reactions, a very low fluence response and low fluence response, are induced by low fluence light (Mandoli and Briggs, 1984). We therefore cannot reject the possibility that the flash induced such reactions and affected the diurnal rhythm of flower opening, although the period of light exposure was very short. To clarify these issues, we need to adopt a method to observe flower opening in darkness without light from now on.

So which endogenous mechanism governs this rhythmic opening of rose flowers? Flower opening is mainly due to the expansion of petal cells with water influx (Evans and Reid, 1988; Kenis et al., 1985; Koning, 1984), and sugar accumulation in petal cells is thought to be a mechanism to reduce petal water potential (Ho and Nichols, 1977). With regard to the function of carbohydrate in the diurnal rhythm of rose flower opening, Evans and Reid (1988) reported that the total carbohydrate content of the petals remained constant during a light-dark cycle, indicating that it is important for maintaining cell size, but it is not the factor controlling rhythmic opening. Resistance to water movement or inadequate water potential gradient along the transport pathway leads to limited water uptake, and the cell-wall strength also limits cell enlargement. In tulip, petal opening and closure occur concomitantly with water transport and are regulated by reversible phosphorylation of aquaporins (Azad et al., 2004). In addition, we have shown that cell-loosening proteins, xyloglucan endotransglycosylase/hydrolase (XTH) and expansions are important in petal growth (Yamada et al., 2007, 2009). The functions of these proteins in rhythmic flower opening will be an intriguing theme hereafter.

Although many studies have focused on senescence to improve the vase life of flowers (van Doorn and Woltering, 2008), few have investigated the physiological aspects of flower opening. The vase life of cut roses depends at least in part on flower opening. Therefore, understanding the mechanism of rose-flower opening is necessary to improve the flower quality and vase life of cut flowers. In this study, we showed that light conditions greatly affected the opening of cut rose flowers without leaves, and that rose petals could perceive light and synchronize their growth to photoperiods. It will be interesting to verify the effect of light wavelength and photoperiod on the vase life and quality of cut roses.

**Acknowledgements**

We would like to thank Prof. Shohei Yamaki of Chubu University for his professional advice and Mr. Masahiro Maesaka of Togo field, Nagoya University for his technical support.

**Literature Cited**

Azad, A. K., Y. Sawa, T. Ishikawa and H. Shibata. 2004. Phosphorylation of plasma membrane aquaporin regulates temperature-dependent opening of petal petals. Plant Cell Physiol. 45: 608–617.

Bielecki, R., J. Elgar, J. Heyes and A. Woolf. 2000. Flower opening in Asiatic lily is a rapid process controlled by dark-light cycling. Ann. Bot. 86: 1169–1174.

Doi, M., M. Miyagawa-Namao, K. Inamoto and H. Inamishi. 1999. Rhythmic changes in water uptake, transpiration and water potential of cut roses as affected by photoperiods. J. Japan. Soc. Hort. Sci. 68: 861–867.

Evans, R. Y. and M. S. Reid. 1986. Control of petal expansion during diurnal opening of roses. Acta Hort. 181: 55–63.

Evans, R. Y. and M. S. Reid. 1988. Changes in carbohydrates and osmotic potential during rhythmic expansion of rose petals. J. Amer. Soc. Hort. Sci. 113: 884–888.

Faragher, J. D., S. Mayak, T. Tirosch and A. H. Halevy. 1984. Cold storage of rose flowers: Effects of cold storage and water loss on opening and vase life of ‘Mercedes’ roses. Sci. Hortic. 24: 369–378.

Hendel-Rahmanim, K., T. Masci, A. Vainstein and D. Weiss. 2007. Diurnal regulation of scent emission in rose flowers. Planta 226: 1491–1499.

Ho, L. C. and R. Nichols. 1977. Translocation of C-sucrose in relation to changes in carbohydrate content in rose corollas cut at different stages of development. Ann. Bot. 41: 227–242.

Ichimura, K. and K. Suto. 1998. Environmental factors controlling flower opening and closing in a *Portulaca* hybrid. Ann. Bot. 82: 67–70.

Jones, M. B. and T. A. Mansfield. 1975. Circadian rhythms in plants. Sci. Prog. Oxford 62: 103–125.

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

Kaihara, S. and A. Takimoto. 1981a. Effects of light and temperature on flower-opening in *Pharbitis nil*. Plant Cell Physiol. 22: 215–221.

Kaihara, S. and A. Takimoto. 1981b. Physical basis of flower opening in *Pharbitis nil*. Plant Cell Physiol. 22: 307–310.

Kenis, J. D., S. T. Silvente and V. S. Trippi. 1985. Nitrogen metabolism and senescence-associated change during growth of carnation flowers. Physiol. Plant. 65: 455–459.

Ichimura, K. and K. Suto. 1998. Environmental factors controlling flower opening and closing in a *Portulaca* hybrid. Ann. Bot. 82: 67–70.
Koning, R. E. 1984. The role of plant hormones in the growth of the corolla of Gaillardia grandiflora (Asteraceae) ray flowers. Amer. J. Bot. 71: 1–8.

Lumsden, P. J. 1991. Circadian rhythms and phytochrome. Annu. Rev. Plant Physiol. Plant Mol. Biol. 42: 351–371.

Mandoli, D. F. and W. R. Briggs. 1984. Fiber optics in plants. Sci. Am. 251: 90–98.

van Doorn, W. G. and U. van Meeteren. 2003. Flower opening and closure: a review. J. Exp. Bot. 54: 1801–1812.

van Doorn, W. G. and E. J. Woltering. 2008. Physiology and molecular biology of petal senescence. J. Exp. Bot. 59: 453–480.

Yamada, K., M. Ito, T. Oyama, M. Nakada, M. Maesaka and S. Yamaki. 2007. Analysis of sucrose metabolism during petal growth of cut roses. Postharvest Biol. Technol. 43: 174–177.

Yamada, K., R. Takahashi, C. Fujitani, K. Mishima, M. Yoshida, J. C. Daryl and S. Yamaki. 2009. Cell wall extensibility and effect of cell-wall-loosening proteins during rose flower opening. J. Japan. Soc. Hort. Sci. 78: 242–251.