論文の内容の要旨

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論文題目

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）

Many ornamentals show flower opening and closing oscillation of diurnal rhythm. 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 Gentianaceae is native to a warm regions of the Southern United States, Mexico, Caribbean and northern South America. *E. grandiflorum* was introduced into Japan more than 60 years ago. Since then, many cultivars having a wide diversity of colors, shapes, and sizes have been released. Thus, it has become a promising flower in the cut flower market in Japan and in the world. *E. grandiflorum* and model plant *Solanum lycopersicum* flowers open in the morning and close at dusk for several days. 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* and model plant tomato.

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.

1. The effect of light on *E. grandiflorum* flower opening and closing oscillation rhythm

In this chapter, 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.

To sum up, *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.

2. The effect of red/far-red in diurnal opening and closing in flowers of *E. grandiflorum*

In order to find out the regulating action of red light photoreceptors (phytochromes), we investigated the effect of red light and/or far-red light on flower opening and closure in *E. grandiflorum*. The results showed that the flower showed normal opening and closing rhythm under different light conditions. The circadian amplitudes are obviously decreased in far-red /dark with 16L/8D condition but not in both red/dark and far-red red/dark conditions. Flowers exhibited differed response on condition of revering the red and far-red light. Flowers 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 up to the fourth day.

Form these results, we found that 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.

3. Phytochromes and cryptochromes together control the diurnal rhythm of flower opening and closing in *Solanum lycopersicum*

In order to clarify to the role of photoreceptors further, we also performed the different light period in model plant tomato with various mutants.

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.

In summary, tomato *S. lycopersicum* flower showed 24-hour cycles of flower opening and closing oscillation rhythm, 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 continuous dark condition. Cry1 located downstream of phyA has been reported. Thus, 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.