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SHORT COMMUNICATION

Effects of light and low temperature on the reciprocal style curvature of Flexistylous *Alpinia* Species (Zingiberaceae)

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Abstract The style curvature (flexistyly) of *Alpinia* species in ginger family is a unique plant organ movement because the style of each flower curves twice during its 1-day anthesis and styles of two phenotypes of each *Alpinia* species in the same population synchronously curve in opposite directions at the same time. In this study, we investigated the effects of low temperature and light conditions on these reciprocal style movements. Our results indicate that low temperature cannot change the direction of each curvature movement, but can slow down these movements and decrease the curve degrees. Light did not affect the upward curvature of the cataflexistylous morph, but the degrees of downward curvature decreased in darkness. For the anaflexistylous morph, the downward curvature only occurred in darkness, but curved directly upward in light condition; after the first (downward) curvature, the second (upward) movement only occurred in light, but did not occur if styles maintained in darkness. These results suggest that low temperature does not stimulate style curvature; light is the necessary condition for the upward movement of the anaflexistylous morph. The stimuli that induced curvature movements in the two morphs were different. Both two curvatures of the cataflexistylous style and downward movement of the anaflexistylous style were controlled via an endogenous program, while the upward movement of the anaflexistylous style was controlled by light.

Keywords Style curvature · Flexistyly · Light · Temperature · Nastic movement · Endogenous program · Photonasty

Introduction

Flexistyly is a novel style curvature movement found in *Alpinia* and *Amomum* (Zingiberaceae) plants. These style movements allow the stigma to avoid being pollinated by its own pollen when the anthers are in dehiscence (Cui et al. 1996; Li et al. 2001). Each flexistylous species has two morphs—an anaflexistylous (protogynous) morph (ana-morph) and a cataflexistylous (protandrous) morph (cata-morph). The style of each morph curve twice within a 1-day flowering period, but the styles of the two morphs curve in opposite directions at the same time (synchronous reciprocal movements) (Fig. 1). For ana-morph individual, when the flower opens at 06:00, the style is elongating and curving downward first, and the anthers are still closed. The second curvature is upward, beginning at 11:00, after which the stigmas move away from the pollinator's foraging pass and the anthers dehisce at 14:00. The style of cata-morph moves in opposite direction. The first curvature is upward, and the anthers dehisce in the morning. The second style curvature is downward and begins at 12:00, allowing the stigma to be pollinated. Flowers of both morphs wilted at 20:00. These unique style movements may promote out-crossing (Li et al. 2001) as well as prevent sexual interference (Sun et al. 2007). The movement of style represents a new reproductive strategy that

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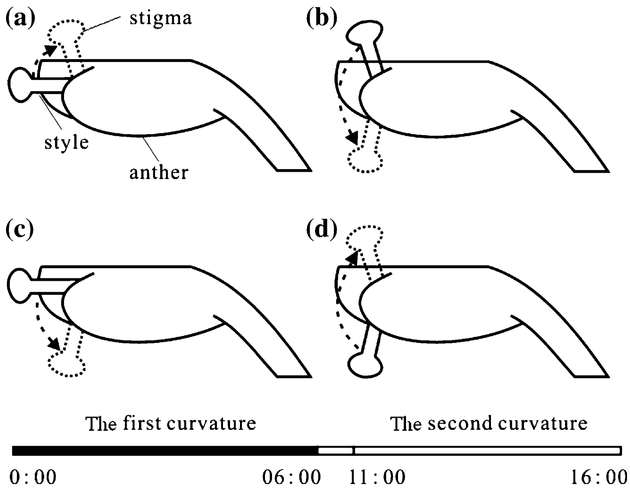

Fig. 1 A sketch of style curvatures in *Alpinia* species. Anthers are held in the same position throughout the 1-day flowering period but the front part of style curved twice: either curved upward first then downward, or curved downward first then downward. **a**, **c** The first curvatures of the cata-morph and the ana-morph, respectively; **b**, **d** the second curvatures of the cata-morph and the ana-morph. The *arrows with a dotted line* indicates the direction of style movements. The *solid bar* denotes night, and the *open bar* denotes day

combines both reciprocal herkogamy and heterodichogamy (Renner 2001; Barrett 2002).

Style curvature is a common phenomenon in flowering plants that has been recognized for a long time, e.g., in Malvaceae (Klips and Snow 1997; Seed et al. 2006), Passifloraceae (Souza et al. 2002), and Marantaceae (Kennedy 1978). In these floral movements, the styles move to one direction after they are stimulated by certain factors or at a certain flowering stage. However, the flexistylous movement of *Alpinia* is distinct from those style curvatures. First, the styles of both morphs of each species curve in opposite directions at the same time. Second, each style curves twice within a 1-day flowering period. Among all floral organs with the ability to move, only flexistylous species display two morphs within a population with styles that move in opposite directions. In our pilot experiments, we found that the changes in gravity vector, the removal of stigmas and/or anthers, stimulation with touching, and pollination did not affect the movements of the style. But in the field, we also noticed that during the days with low temperature (compare to normal weather), the flexistylous movements were abnormal. It also remains to be elucidated how the light conditions affect these reciprocal style curvatures.

In this study, we attempted to answer two questions: (1) do low temperature and light conditions influence flexistylous curvatures of *Alpinia* plants? (2) if so, whether these environmental factors have the same impacts on two morphs' reciprocal movements?

Materials and methods

Plant material

Alpinia platychilus K. Schumann was used in this study. It is a perennial herb, usually 2–5 m tall. The racemes are erect on the terminal ends of leafy shoots. The flowers have typical ginger floral structures. A conspicuous three-lobed labellum is produced by the fusion of two staminodes, and is yellow tinged with red and obovate. One fertile stamen with two anthers develops, and the style extends between the anthers. During the flower season, one inflorescence has one to five flowers synchronous bloom each day, and a single flower only lasts 12 h.

Sampling strategies

Since plant size of the research species is too bigger to be controlled, we only use detached flowers in the experiments. Moreover, for the convenience of observation and measurement, we removed most of the floral structures, only remained male and female organs, including ovary, style and filament (Fig. 2). Our previous experiments indicated that with an adequate water supply and the maintenance of high humidity, the style of detached flower without corolla lobes and labellum, has the same curvature movement rhythm as that of flowers on mother plants.

Low temperature treatments

To detect the effects of low temperature on the two style curvatures of each flexistylious morphs, we sampled *Alpinia platychilus* flowers at two floral development stages: (1) at 21:30 of the day before the flower opened (before the first curvature); and (2) at 6:00 of the flowering day (right after the first curvature and before the second curvature). After the flowers were sampled, the tubular calyx, corolla lobes and labellum were removed, and the remaining parts including style were put in bottles with deionized water and placed in growth chambers with low temperatures of 4, 15°C or normal room temperature (RT, about 25°C, almost same with the normal field weather condition), respectively. The light conditions were corresponded with the field at the real time. We recorded the stigma–anther angle once in 1-h intervals.

Light treatments

To detect whether light conditions influence the different curvatures of two style morphs, the flowers were sampled at three floral development stages: (1) before the first curvature, at 22:30, 1 day before flower anthesis; (2) right after the first curvature, at about 06:00 (before dawn) on

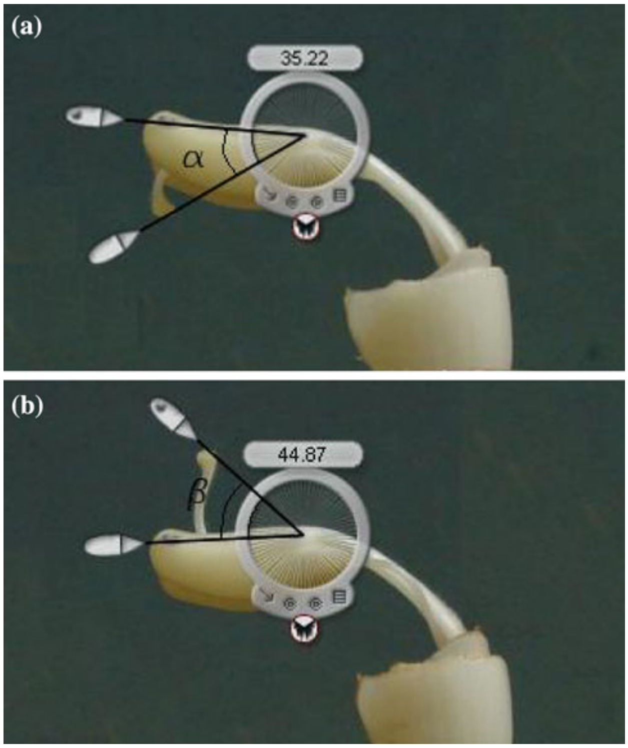

Fig. 2 Method for measuring the relative stigma–anther position. One side of a protractor was overlapped with a line defined by two points protruding from one lateral side of the anther. The vertex of the protractor was the point that was close to the filament. Another side of the protractor comprised the vertex and stigma. The acute angle measured by the protractor was denoted α or β. When the stigma was under the anther, the stigma-anther angle was 180-α; when the stigma was over the anther, the stigma-anther angle was 180 + β

the day of flowering; and (3) before the second curvature, at about 10:30 (after 4–5 h of daylight). After the flowers were sampled, the tubular calyx, corolla lobes, and labellum were removed, the remaining parts including style were placed in growth chambers with dark or light conditions (white light, 200 μmol $m^{-2}s^{-1}$), the temperature was same with the field (25°C). We then recorded the stigma–anther angle before and after the treatments.

Determination of style curvature

We used the change of the angles between anther's axle wire and the line from stigma to the end part of filament to represent the extent of style curvature, or the relative change of stigma-anther angle. The angles were measured by Screen Protractor software (version 4.0, Iconico, Inc., http://www.iconico.com) (Fig. 2). If the style curved upward, the style curvature was positive; if the style curved downward, the curvature was negative. Therefore, the angles of the first curvatures of anaflexistylous style (α) and cataflexistylous style (β) were negative and positive, respectively. The second curvature of anaflexistylous style (α + β) and cataflexistylous style (β + α) were positive and the negative, respectively.

Results and discussion

Effect of low temperature on style curvature

Plant organ movement is usually influenced by environmental temperature; for example, at a constant low temperature, *Oxalis martiana* flowers do not open (Tanaka et al. 1989) and young *Phryma leptostachya* primary stems remain prostrate (Endo and Miyauchi, 2006). Low temperature-mediated inhibition of plant organ movement has also been reported in the gravitropism of inflorescence *Arabidopsis* stems (Wyatt et al. 2002) and the phototropism of *Arabidopsis* seedlings (Orbović and Poff 2007). Our results showed that the curvature movements of both style morphs of *Alpinia platychilus* were influenced by low temperature: the rate of style curvature movements were positively correlated with the environment temperature. The degrees of the full curvature under different temperature also showed low temperature decreasing the degrees of curvatures; for instance, at RT, the first curvature degree of ana-morph was −29.02 ± 6.47°, but that decreased to −18.27 ± 4.35° and −5.83 ± 3.26° at 15 and 4°C, respectively (Table 1); the second curvature degrees were 30.09 ± 3.27°, 45.65 ± 3.88°, and 58.22 ± 5.24° at 18:00 when the flowers were placed at 4, 15°C, and RT, respectively (Fig. 3a). Low temperature had the same effect on the cata-morph (Table 1; Fig. 3b). These findings indicate that flexistylous style curvature did not belong to thermonastic movement (defined as plant movement corresponding to a change in temperature) (Tanaka et al. 1989). The results of low temperature impact on the flexistylous movements may attribute to the effects of low temperature on auxin synthesis, transportation, or metabolism, serving to delay the response of the tissue to incoming auxin and decrease the speed and bending rate of curvature. In addition, low temperature may decrease membrane fluidity and diminish auxin carrier movement, thereby interfering with sensation and decelerating the redistribution of auxins.

Effect of light on style curvature

Light is another factor that plays an important role in plant movement (Darwin 1880; Hart 1990; Gilroy and Masson 2008). Maize roots show no gravitropic curvature when grown in darkness (Suzuki et al. 1981; Feldman and Briggs 1987). Light may influence both photochemical transformation of a photoreceptor and NADPH/NADP ratio. In addition, light plays an essential role in promoting auxin

Table 1 Effects of temperature on style curvature in *A. platychilus*

Phenotype	Temperature (°C)	Degree of the first curvature (°)	Degree of the second curvature (°)
Ana-morph	4	-5.83 ± 3.26^{a}	30.09 ± 3.27^{a}
	15	-18.27 ± 4.35^{b}	45.65 ± 3.88^{b}
	RT	-29.02 ± 6.47^{c}	58.22 ± 5.24^{c}
Cata-morph	4	5.24 ± 2.47^{a}	0^{a}
	15	26.46 ± 5.22^{b}	-16.21 ± 6.29^{b}
	RT	38.29 ± 3.96^{c}	-56.28 ± 5.35^{c}

All styles were maintained in a room with a constant temperature of 4°C, 15°C or RT ($n = 15$). The first curvature occurred in darkness, and the second one in light. Values marked with different letters are significantly different according to the multiple Duncan Test, $P < 0.05$; $n = 15$

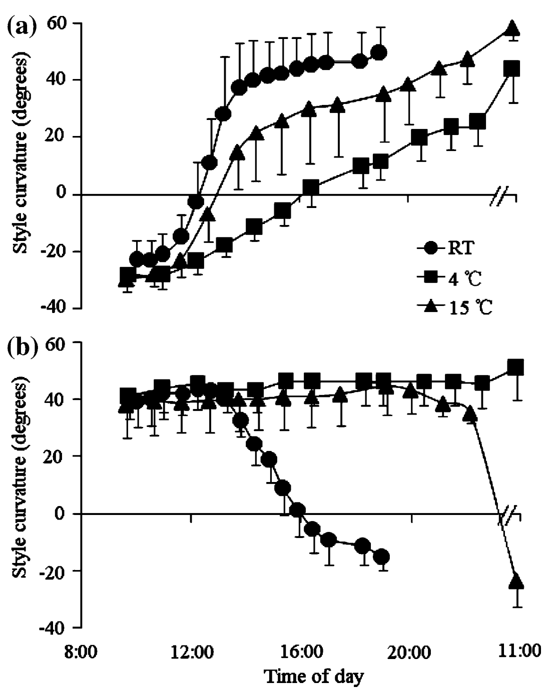

Fig. 3 Effect of low temperature on the second style curvatures of *A. platychilus*. **a** Ana-morph ($n = 15$). **b** Cata-morph ($n = 15$). The flowers were placed at 4, 15°C or RT at 06:00, and the stigma–anther angle was then measured once an hour. Although movement was slow at lower temperature, the styles ultimately completed the entire process excluding cataflexistylous styles placed at 4°C

polar transport during gravitropic bending (Scott and Wilkins 1969; Laxmi et al. 2008).

The influence of light on flexistylous movement is more complicated, depending on the phenotype of style and their development stages. For the first curvature, the cataflexistylous style could complete upward curvature both in darkness and in light with the magnitudes of $38.57 \pm 0.27°$ and $37.37 \pm 4.13°$, respectively (Fig. 4), and no significant difference between them (t test, $t = 1.73$, $P > 0.05$). But the first (downward) curvature of ana-morph style only occurred in darkness (natural conditions); light will induce the style curving upward instead

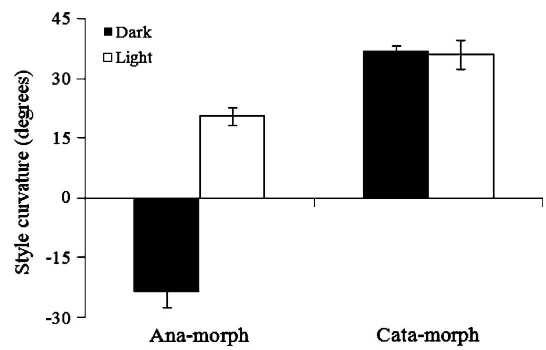

Fig. 4 Effect of light on the first curvature of *A. platychilus*. Styles were treated at about 21:00. We then removed all parts of the flower except the stamen and pistil and placed the base into absorbent cotton saturated with deionized water. The magnitude of style curvature in the ana-morph under light conditions was significantly greater than in the dark ($t = 7.867$, $P = 0.00$). The directions of curvature in darkness and in light were opposite. No differences were observed in the cata-morph between dark and light conditions in either magnitude or direction ($t = 0.110$, $P = 0.914$)

of downward (Fig. 4). The anaflexistylous style curved downward about $-23.14 \pm 6.50°$ in darkness and upward about $16.99 \pm 3.51°$ in light. There was a significant difference between the curvatures in darkness and in light.

The extent of the second style curvature depended upon the time of light treatment. The styles of the cata-morph that were sampled before dawn (after the first curvature) curved more in light than in darkness (t test, $t = 3.14$, $P < 0.05$; Fig. 5a). But when the styles were sampled after being exposed to light for 4 h, there was no difference in the degree of style curvature between the light and dark conditions (t test, $t = 1.23$, $P > 0.05$; Fig. 5b). The effect of light on the second curvature of the ana-morph was different from the effect on that of the cata-morph. If styles were sampled before dawn and maintained in darkness, the styles only curved upward a small degree (Fig. 5a); however, complete upward curvature occurred when the sampled styles were kept in light (t test, $t = 10$, $P < 0.01$; Fig. 5b). As in the cata-morph, the style curvatures of ana-morph were the same

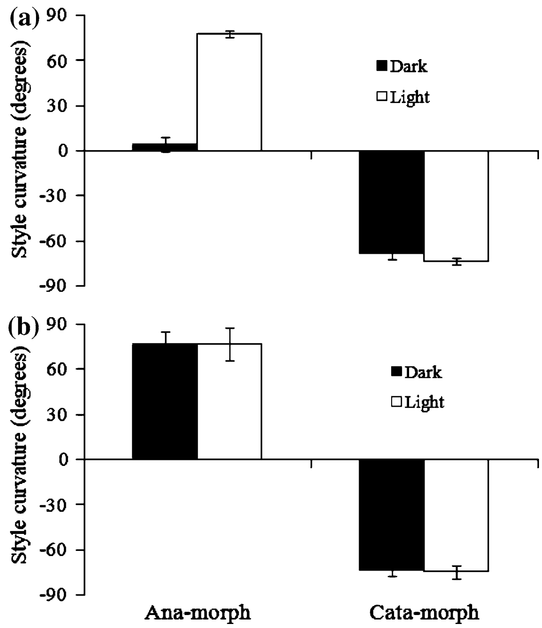

Fig. 5 Effect of light on the second curvature of *A. platychilus*. **a** The flowers were picked before dawn and immediately placed into either darkness ($n = 20$) or light ($n = 20$). The magnitudes of style curvature were significantly greater for both morphs under light conditions than under dark conditions (ana-morph, $t = -4.973$, $P = 0.016$; cata-morph, $t = -0.5.871$, $P = 0.028$). **b** Flowers were placed in darkness at 11:00 ($n = 20$). There were no differences in the magnitude of style curvature for either morph under the dark or light conditions (ana-morph, $t = 1.103$, $P = 0.277$; cata-morph, $t = -0.683$, $P = 0.499$)

in darkness and in light if treatments was performed after exposure to light for 4 h (t test, $t = -9.81$, $P > 0.05$). Further experiments indicated that 30 min of illumination (placed in darkness after illumination) is enough to induce the styles of ana-morph to move upwards, while a flash-light or a shorter period of illumination (<30 min) does not lead to the corresponding movement.

We divided the flowering period into three phases, 00:00–06:00 (before the first curvature), 06:00–11:00 (before the second curvature), and 11:00–18:00 (ongoing of the second curvature). We treated styles with all possible combinations of light and darkness treatments. The results indicate that the patterns of the ana-morph could be divided into two parts: (1) the styles moved downwards if placed in darkness at 00:00–06:00 (Fig. 6a); (2) the styles moved upwards if placed in light at 00:00–06:00, like the normal curvature of cata-morph (Fig. 6b). The patterns of cata-morph were similar under different light–dark conditions (Fig. 6c, d), i.e., the styles move upwards and then downwards.

The different effects of light on flexistylous movements indicate that the stimuli leading to style curvature are different for the two phenotypes, and the mechanisms of curvature may also be different. The Cholodney–Went hypothesis suggests that the asymmetric distribution of auxin induces the curvature of some organs (Went and Thimann 1937). Because each flexistylous style has two reciprocal curvatures, auxin transport may be involved in

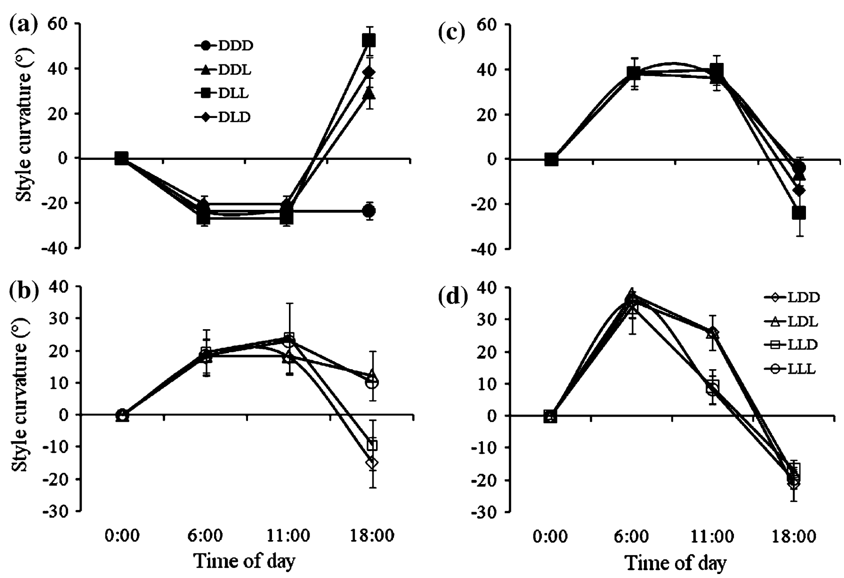

Fig. 6 The curvature of styles of *A. platychilus* under different combinations of light and dark conditions. Flowers were placed in bottles with water after removing the bractlets, corolla, and labellum at 23:00. We recorded the stigma-anther angle at 00:00, 06:00, 11:00 and 18:00 and defined the interval from 00:00 to 06:00 as phase I, from 06:00 to 11:00 as phase II, and from 11:00 to 18:00 as phase III. We use "D" or "L" to represent darkness or light at each phase, respectively. Thus, "DDD" indicates that phase I, II, and III were all in darkness, and so on. In order to make the data easier to follow, we organized the treatments that were in darkness during phase I into a series (the D-series) and the treatments that were in light during phase I into another series (the L-series); **a** ana-morph D-series, **b** ana-morph L-series, **c** cata-morph D-series, and **d** cata-morph L-series

the curvature process. Light can promote auxin transport by regulating auxin efflux carriers during gravitropic curvature (Laxmi et al. 2008). It is possible that light plays a similar role in style curvature in *Alpinia* species, but the actual role of light requires further investigation.

In conclusion, above freezing low temperature does not stop style curvatures, but it does affect the rate of movements by influencing the growth rate of plant tissue. Light is not necessary for the curvatures of cataflexistylous style; however, it controls curvatures of the anaflexistylous style. The style curvature of the cata-morph is controlled via an endogenous developmental program. The first curvature of the ana-morph style is also controlled by an endogenous program, while the second curvature may be photonastic movement. However, elucidation of the reason why light affects similar curvature behaviors in different ways requires additional investigations.

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