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Floral Initiation, Organogenesis, and Flowering of Mature *Paphiopedilum* Clair de Lune 'Edgard Van Belle' Award of Merit/American Orchid Society Plants Derived from Divisions

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***Abstract. Paphiopedilum* Clair de Lune 'Edgard Van Belle', an excellent Maudiae-type hybrid that has been propagated by artificial division for a long time. We studied its flower bud initiation, development of floral organs, and flowering habits with a view to providing information for flowering control and efficient commercial production. According to our research, the flower bud initiation phase of this cultivar begins in February every year, and 80% of the plants completed sepal primordium differentiation in March, The flower bud differentiation lasts for 6 to 7 months, until flowering in August. Within 1 to 3 months after flower bud differentiation, all tested plants differentiated lateral buds. After 5 to 6 months, the new, aboveground vegetative shoots reached their maximum growth, with an average plant height of 20 cm, five leaves, and a shoot dry weight of more than 3 g. From February to April of the following year, a new cycle of flower development and vegetative growth began. In addition, this cultivar was notably sensitivity to high ambient temperature during the late phase of flower development, with a flower bud drop rate as high as 33.3% under average day/night temperatures of 29.0/26.5 °C.**

The genus *Paphiopedilum*, commonly known as the lady's slipper orchid, consists of more than 98 terrestrial species (Koopowitz 2000), with a few epiphytic or lithophytic species, that are distributed from India and southwestern China to Southeast Asia, the Malaysian islands, and the Solomon Islands (Cribb 1998). *Paphiopedilum* species exhibit a diverse altitudinal distribution, spanning from sea level, such as *Paphiopedilum godefroyae*, to elevations as high as 2000 m, such as *Paphiopedilum armeniacum*. Because of the long-lasting flowers, *Paphiopedilum* hybrids are commonly used as potted plants and for the production of cut flowers. In commercial production of orchids (e.g., phalaenopsis and cymbidium), being able to control flowering time allows nursery growers to align their production with market demand for specific seasons or events. Understanding the physiological mechanisms controlling flowering is critical to improve the success in manipulating flowering in the horticultural market.

The transformation of a shoot apical meristem (vegetative phase) into an inflorescence meristem (reproductive phase), forming bracts and flower buds, is a morphologically complex shift in plant development (Benlloch et al. 2007; Henderson and Dean 2004; Liu et al. 2009; Thouet et al. 2012). Floral transition depends on both endogenous and environmental factors, including different pathways (e.g., photoperiod, vernalization, hormonal and autonomous age-related pathways) (Amasino 2010; Blázquez et al. 2003; Mouradov et al. 2002; Mutasa-Göttgens and Hedden 2009). In several orchids, temperature plays a critical role among the factors affecting floral transition (Lopez and Runkle 2005). In *Phalaenopsis*, high temperatures (>28 °C) inhibited floral induction significantly (Sakanishi et al. 1980). Flowering of *Phalaenopsis amabilis* was induced because the plants were grown at cool temperatures (25/20 °C day/night) compared with high temperatures (30/23 °C day/night) (Chen et al. 1994). Similarly, relatively high temperatures may be disadvantageous to floral induction in *Dendrobium nobile* and potted *Miltoniopsis* (Lopez and Runkle 2006; Yen et al. 2008). To the best of our knowledge, there few studies on the growth and flower development of *Paphiopedilum* orchids (Feng et al. 2021; Pi et al. 2009; Yin et al. 2022).

Paphiopedilum species have sympodial growth, and the shoot apical meristem may develop into an inflorescence or it may be aborted. In *P. armeniacum* (*Parvisepalum* subgen.), flower bud differentiation initiates in May, with full development of all floral organs by mid August (Pi et al. 2009). Furthermore, flower bud differentiation in a spring-flowering species [*Paphiopedilum micranthum* (*Parvisepalum* subgen.)] and in two autumn-flowering species [i.e., *Paphiopedilum dianthum* (the section Pardalopetalum) and *Paphiopedilum henryanum* (the section Paphiopedilum)] all started in April and May (Feng et al. 2021). These results indicate that the variation in flowering time among some *Paphiopedilum* species could be the duration of floral bud development, as opposed to floral bud differentiation timing (Feng et al. 2021).

Paphiopedilum encompasses a diverse group of species across various phylogenetic clades (Cribb 1998), and they can be found in various habitats, from seashores to high mountains. Lowland species may prefer warmer conditions, whereas mountain species can tolerate cooler temperatures, suggesting species-specific flowering requirements. Previous studies (Feng et al. 2021; Pi et al. 2009) incorporated species from the subgenus *Parvisepalum*, sections Pardalopetalum and Paphiopedilum, with a predominant distribution in southwestern China. In the horticultural market, a popular *Paphiopedilum* hybrid known as the "Maudiae type" is derived from the hybridization of species found in the section Barbata, and primarily inhabits the tropical Pacific islands. In this study, we investigate whether a summer-flowering Maudiae-type hybrid, *Paphiopedilum* Clair de Lune 'Edgard Van Belle' is influenced by the onset of flower bud differentiation or by the duration of this developmental process.

In previous studies (Hsiao and Lin 2013; Pi et al. 2009) of the flowering of *Paphiopedilum*, investigations were conducted using wild or sexually propagated seedlings. However, as a result of the presence of a heterozygous genetic background, it is difficult to maintain consistent uniformity among experimental materials.

In our study, we used division-derived plants of *Paphiopedilum* Clair de Lune 'Edgard Van Belle' as the experimental material. This clonally propagated cultivar makes it a suitable experimental material for investigating the timing of floral development and flower phenology of *Paphiopedilum*. In practical horticulture, a better understanding of flower development and flower phenology will provide more efficient flowering control and greater quality *Paphiopedilum* flower production.

Materials and Methods

Plant materials and culture conditions. *Paphiopedilum* Clair de Lune 'Edgard Van Belle', a selected, elite Maudiae-type hybrid created by crossing the seed parent (*Paphiopedilum* Emerald) with the pollen plant (*Paphiopedilum* Alma Gevaert), was used as the experimental material. The experimental plants were propagated through long-term division, with one or two bloomed shoots (back shoots) and one developing young shoot bud (current shoot, labeled shoot A in Fig. 1), and were planted in 3.5-inch plastic pots containing a mixture of one part bark (5–12 mm) and one part pumice stone (7–15 mm). The plants were grown in a greenhouse with a fan and pad cooling system under ambient light (70% shade) at Miao Hua Orchids Garden (Xiushui Township, Changhua County, Taiwan). The temperature during the experimental period was collected using a HOBO U12-011 data logger (Smartec Scientific Corp., New Taipei City, Taiwan) (see Supplemental Fig. 1). Plants were watered once or twice a week, depending on the climate, and were fertilized with a slow-release fertilizer containing 13N–11P–13K–2 trace elements (Hi-Control® S-101 Type 180; Asahi Kasei, Tokyo, Japan) once before March each year.

Observation of floral transition, plant growth, and data collection. From 19 Feb 2017 to 21 Dec 2018, the shoots of five plants (Feb 2017–Mar 2018) or three plants (May 2018–Sep 2018) were collected randomly and dissected every month to determine their developmental phase and to calculate the differentiation of flower bud and lateral bud. Because sympodial orchids form new lateral buds as the plant grows, we labeled the oldest to youngest shoots A to C, respectively (Fig. 1), as markers for experimental sampling. The differentiation of floral primordia and organs was observed and photographed with a stereo microscope (Nikon SMZ800; Nikon, Japan) equipped with a monocular digital camera (Nikon D5100; Nikon). In total, 360 plants were used in our experiment.

In addition to floral transition, plant height (measured on the current shoot from the cultivation medium surface to the highest point, where the leaves naturally unfold), leaf number (total number of leaves on the current shoot), shoot dry weight (the dry weight of the leaves and stem without the flower parts after drying in an oven at 70 °C for 48 h), root length (the length of the longest root), root number (the number of roots longer than 0.2 mm), root dry weight (the root parts after drying in an oven at 70 °C for 48 h), flower stalk length (the base of the flower stem to the lower edge of the ovary), flowering rate, and flower bud drop rate were also measured during the experiment. Fifteen plants were selected randomly for assessment every month. The average values and standard

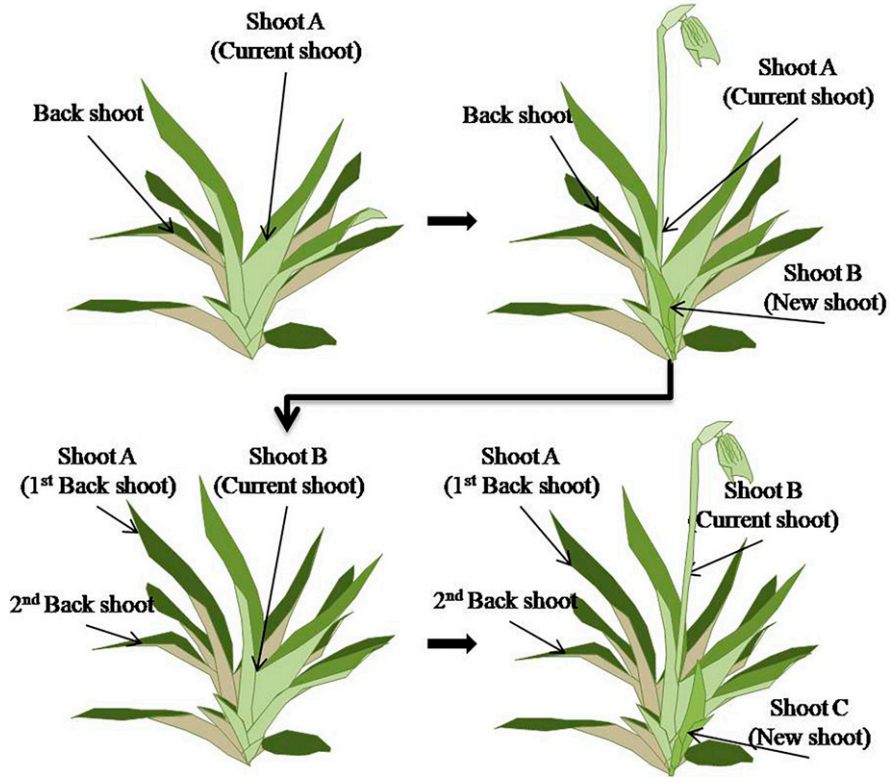

Fig. 1. Schematic diagram of growth morphological changes and experimental sampling of *Paphiopedilum* Clair de Lune 'Edgard Van Belle'. The plants were sampled every month, and the growth and development of shoots A, B, and C were recorded.

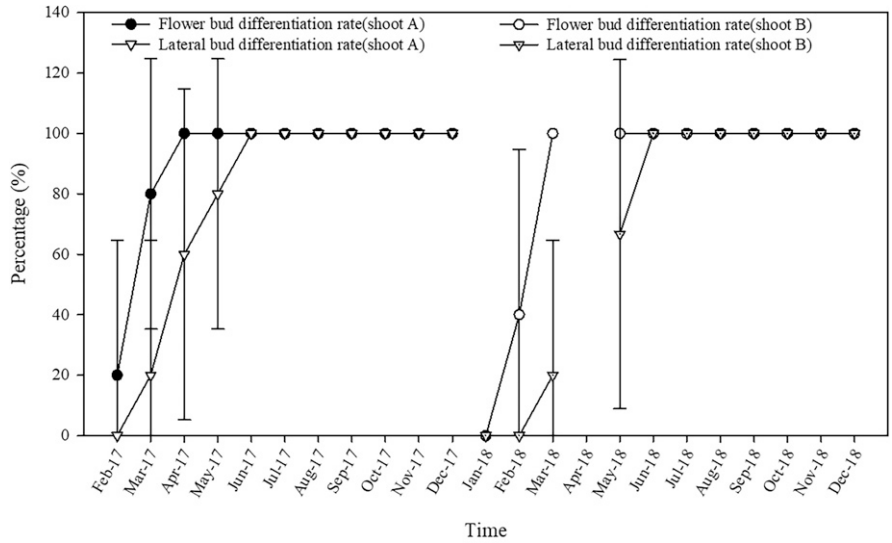

Fig. 2. The percentage of flower bud differentiation and lateral bud organogenesis of *Paphiopedilum* Clair de Lune 'Edgard Van Belle' during two growth cycles.

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deviation (*SD*) are reported. However, the root length, root number, dry weight, and flower stalk length data were derived by destructive sampling from five or three plants used in the morphological observation.

Experimental design and statistics. We used systematic sampling to investigate plant morphological traits. *SD*s were calculated using the descriptive statistics of Excel ver. 2010 (Microsoft Corp., Redmund, WA, USA). Graphic images were produced using Sigma Plot ver. 12.0 (Systat Software Inc., Palo Alto, CA, USA).

Results

Observation of vegetative and reproductive growth cycles in Paphiopedilum *Clair de Lune 'Edgard Van Belle'.* The differentiation of inflorescence primordium and lateral buds of *Paphiopedilum* Clair de Lune 'Edgard Van Belle' was observed monthly using a stereomicroscope for 23 consecutive months. At the beginning of the experiment (Feb 2017), 20% of the shoot apical meristems of the current shoot (shoot A) had already transformed into flower buds. By March, 80% of the apical meristems had converted to flower buds, and by April this transition reached 100%. As the apical meristems transitioned from vegetative to reproductive growth (February to April), lateral buds (shoot B) began to develop at the stem nodes. Approximately 20% of the plants showed lateral bud formation in March and 100% by June. The newly formed lateral buds (shoot B) continued to develop, and in Feb 2018 began to convert to the reproductive phase. New lateral buds (shoot C) formed again in Mar 2018 (Fig. 2), and the growth cycle continued.

Morphology of flower bud and floral organ development. During the floral transition of *Paphiopedilum* Clair de Lune 'Edgard Van Belle', the bracts and inflorescence primordium are formed first, then the flower bud primordium is observed in the bracts. In January, the shoot apical meristem maintains a hemispherical dome-like structure surrounded by leaf primordium (Fig. 3A). From February to April, the shoot apical meristem transitions into a broad, oval inflorescence primordium with two distinguishable flower buds (Fig. 3B). As the central region of the first flower meristem gradually becomes concave, the perianth organs subsequently differentiate from the peripheral ridge (Fig. 3C). After the formation of perianth organs such as the dorsal sepal, synsepal, and three inner perianth lobes, two petal primordia and one lip primordium, pseudostamens, stamens, and gynostemium are subsequently differentiated (Fig. 3D–F). Before blooming at the end of August, the floral organs gradually mature (Fig. 3G and H), eventually leading to the full flowering phase (Fig. 3I).

Progress of vegetative and reproductive growth cycles of Paphiopedilum *Clair de Lune 'Edgard Van Belle'.* The results of continuous growth observation for 2 years for *Paphiopedilum* Clair de Lune 'Edgard Van Belle' were mapped as a developmental timeline (Fig. 4). From March to June each year, to February to April of the following year, the new

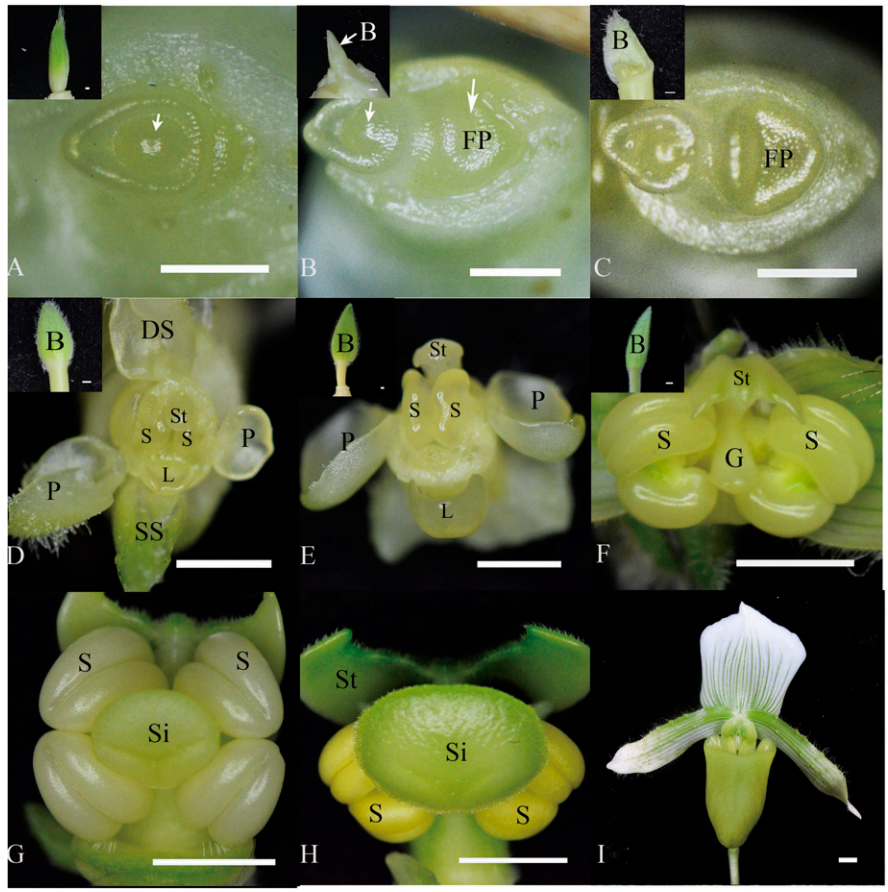

Fig. 3. The morphology of flower bud development in *Paphiopedilum* Clair de Lune 'Edgard Van Belle'. (**A**) Shoot apical meristem before the beginning of flower initiation in January. The arrow indicates the dome. The inset in the upper left shows the outer leaves of the observation target. Bar = 0.5 mm. (**B**) The flower primordium forms in February. Arrows indicate the two flower buds. Bar = 0.5 mm. (**C**) The floral primordia differentiate in bracts, and floral organs begin to form in March. Bar = 0.5 mm. (**D** and **E**) Morphology of floral organs development from April to May. Bar = 1 mm. (**F**) In June, the gynostemium and ovary gradually differentiated and developed. Bar = 3 mm. (**G**) Morphology of floral organs when the flower stalk grew rapidly in July. Bar = 3 mm. (**H**) Flower buds mature and begin to bloom in August. Bar = 4 mm. (**I**) Flowering in September. Bar = 1 cm. B = bract; FP = flower primordium; DS = dorsal sepal; G = gynostemium; L = lip; P = petal; S = stamen; Si = stigma; SS = synsepal; St = pseudostaminode.

vegetative shoot reached physiological maturity and transformed into a flower bud. The entire process lasted ~10 to 11 months. After the flower buds were initiated, they quickly differentiated into inflorescence and flower primordium, then transitioned to floral organ differentiation. The sepal primordium differentiation was complete in March, petal primordium differentiation in April, and gynostemium primordium differentiation in May. Subsequently, the floral organs gradually matured and bloomed in August to September. The entire process took 15 to 16 months (Fig. 4). In addition, because the lateral buds developed immediately after flower bud differentiation, both the flower buds transformed from terminal buds and the newly formed vegetative shoots from the stem nodes grew simultaneously, resulting in annual flowering.

Dynamic changes in plant traits during successive growth cycles of Paphiopedilum *Clair de Lune 'Edgard Van Belle'.* Through the growth curves of shoot- and root-related traits during the successive growth cycle, the relationship between plant growth changes and physiological maturity of *Paphiopedilum* Clair de Lune 'Edgard Van Belle' was explored. At the beginning (Feb 2017), the average plant height of the current shoots (shoot A) was 20.3 ± 2.6 cm. At that time, the plants had reached physiological maturity, and some plants had begun to form flower buds. As the shoot apical meristem transformed into a flower bud and started the reproductive growth phase, there was no significant change in plant height. The new shoots (shoot B) formed in March of the same year, and by Nov 2017, their height had exceeded 20 cm. Thereafter, the plant height never increased. Shoot C showed a similar trend to shoot B in terms of plant height (Fig. 5A).

The reproductive growth phase of the current shoots (shoot A) began when there was an average of 5.3 ± 0.6 leaves. The leaf number remained consistent until flowering, at an average ranging from 5.2 to 6.1 leaves. The new shoots (shoots B and C) fully developed an average of 5.6 ± 0.6 leaves by November, which was close to the time when the maximum plant height was reached (Fig. 5B). In addition, we observed that some plants gained an extra leaf when starting the reproductive

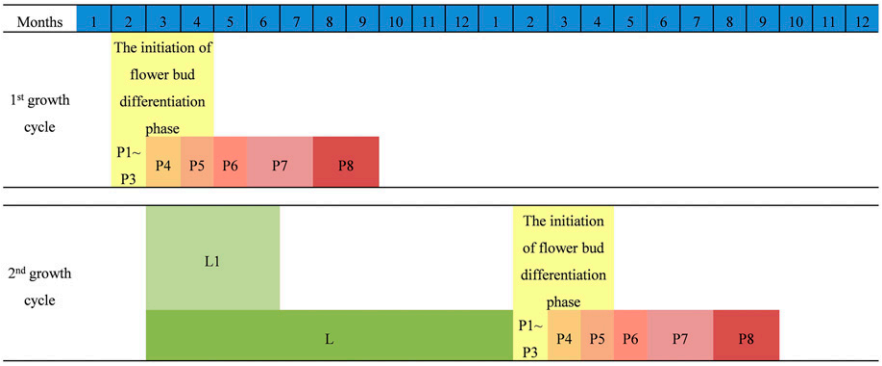

Fig. 4. Timing of vegetative and reproductive growth cycles of *Paphiopedilum* Clair de Lune 'Edgard Van Belle'. Two growth cycles of leaf and flower development were mapped. L = lateral bud growth period; L1 = initiation of lateral bud differentiation phase; P1 = initiation of differentiation phase (because of the different timing of individual plants, the actual differentiation period is also marked as a time range in light yellow); P2 (abbreviation not shown in figure, but included in range) = inflorescence primordium differentiation phase; P3 = flower primordium differentiation phase; P4 = sepal primordium differentiation phase; P5 = petal primordium differentiation phase; P6 = gynostemium primordium differentiation phase; P7 = growth of flower phase; P8 = flowering phase.

growth phase. This is attributed to the slower development of the upper leaf, which gradually wraps around the base of the flower stalk as it elongates, like a sheath leaf.

The mature current shoots (shoot A) had an average dry weight greater than 3.04 ± 1.22 g. As the terminal flower buds developed gradually until flowering, the average dry weight remained between 3.04 and 4.66 g. By March of the following year, when the new shoots (shoot B) started to form flower buds, the average dry weight of shoot A increased to 6.34 ± 2.17 g. The average dry weight of shoot B formed from March to June, reaching 3.51 ± 0.66 g after December, and remained at 3.09 to 4.93 g until flowering (Fig. 5C).

In terms of root development, the average number of roots for the nearly mature current shoots (shoot A) was 3.8 ± 2.8 roots, and the average root length was 4.72 ± 2.57 cm. As the plants began to differentiate flower buds and new lateral buds, the number of roots continued to increase, reaching a maximum average of 8.8 ± 2.6 roots and a length of 20.40 ± 6.84 cm (Fig. 5D and E). The newly differentiated lateral buds (shoot B; from March to June) began to form roots in November, with an initial root weight of 0.12 ± 0.06 g. Shoot C began to develop roots in August of the following year, with an initial root weight of 0.02 ± 0.03 g. This suggests that the development of new vegetative shoots after differentiation in March takes at least 5 to 8 months to initiate root development (Fig. 5F).

Flower phenology and flower bud drop of Paphiopedilum *Clair de Lune 'Edgard Van Belle'.* After the plant reached the reproductive phase, the floral organs differentiated gradually, and the length of the flower stalk increased. However, the flower stalk developed slowly before June, with an average length of no more than 3 cm. It grew rapidly in July and reached an average length of more than 20 cm. When flowering, the maximum length of the flower stalk reached 34.6 to 40.2 cm. *Paphiopedilum* Clair de Lune 'Edgard Van Belle' bloomed in August and September, and had the highest flowering rate of 73.3%. However, during development, more than 26.7% of the flower buds fell off. The dropping of flower buds occurred mainly in the late phase of flower development, from July to September, and the maximum bud drop rate was 33.3% (Fig. 6). There were two main periods and locations that caused flower bud drop. The first occurred during the early phase of rapid elongation of the flower stalk in July. At that time, the flower stalk emerged, but the flower buds were not yet visibly developed or enlarged. Initial browning occurred in the ovary and at the connection between the ovary and the column (Fig. 7A and C). The other period was when the flower stalk was nearing full development, and the flower bud had already enlarged. The initial browning occurred in the stamen (Fig. 7B and D).

Discussion

Morphological transformation of Paphiopedilum *from terminal bud to flower bud.* There are two hypotheses for the morphological characteristics of the terminal buds of *Paphiopedilum* that transform into flower buds (Feng et al. 2021; Pi et al. 2009; Yin et al. 2022). The first explanation suggests that during the initiation of flower bud differentiation, the shoot apical meristem widens and enlarges, first forming a folded, protective sheath-like structure called the inflorescence sheath. Subsequently, the bract primordium emerges, the apical meristem in the bract continues to grow and expand, and two semicircular protrusions become floret primordia that form in the middle, officially converting to the flower primordium differentiation phase (Pi et al. 2009; Yin et al. 2022). Another explanation holds that during the initiation of flower bud differentiation, the shoot apical meristem changes its hemispherical structure gradually to form flattened protuberances and differentiates into the inflorescence primordium. Subsequently, bracts differentiate on both sides of the inflorescence axis, and then the flower primordium differentiates in the bract (Feng et al. 2021). We argue that before the apical meristem starts to widen and flatten, and before differentiation of flower primordium within the bracts, it is not easy to determine whether the protrusions shaped in the peripheral structure of the hemispherical apical meristem are leaf primordium, inflorescence sheaths, or bracts. The so-called inflorescence sheath tissue, which is probably the last leaf that is not fully expanded, varies in size when the plant begins to differentiate flower buds. As the flower bud grows, it elongates gradually. Therefore, in some individual plants, an extra leaf may be observed before the flower stalk elongates. To ensure the consistency of the subsequent discussion on flower bud differentiation, we define the initiation of flower bud differentiation as bracts starting from the inflorescence primordium, and the widening and flattening of the apical meristem.

Flower bud differentiation in Paphiopedilum. A previous study showed (Feng et al. 2021) that mature plants of *P. micranthum*, *P. dianthum*, and *P. henryanum* all initiate flower bud around May, although their flowering periods differ. In *P. armeniacum*, flower bud initiation occurs in May, followed by the formation of the inflorescence sheath in June and July. However, the onset of irreversible flower bud differentiation does not occur until the end of August, when the bracts form. Subsequently, these plants bloom the following February (Pi et al. 2009). *Paphiopedilum callosum* differentiates inflorescence sheath primordium in June and bract primordium in late August (Yin et al. 2022). According to the research just described, we concluded that if the formation of bracts is used as the starting point to determine flower bud initiation, there are two main periods for flower bud initiation of *Paphiopedilum*: February to May and after August. The duration of flower bud differentiation varies with species, with the duration for *P. dianthum* ≈16 to 17 months; for *P. micranthum*, ≈10 to 11 months; for *P. henryanum*, ≈4 to 5 months; for *P. armeniacum*, ≈6 to 7 months; for *P. callosum*, ≈8 to 9 months, and, as shown in our study, for *Paphiopedilum* Clair de Lune 'Edgard Van Belle', ≈6 to 7 months.

We propose the following two hypotheses regarding flower bud differentiation in the genus *Paphiopedilum*. The first is based on the facts that flower bud initiation occurred mainly from February to May (but fully ranging from December to April) and after August (but ranging from June to August), and that orchid flower bud induction needs 4 weeks to 2 months. This coincides with the winter and summer seasons in the northern hemisphere, which exhibit opposite conditions in terms of temperature and daylength. Therefore, *Paphiopedilum* species may have two different sets of requirements for optimal conditions during flower bud induction. The second hypothesis takes into consideration 1) the vegetative growth status of the plants and 2) the suitability of the environmental conditions during

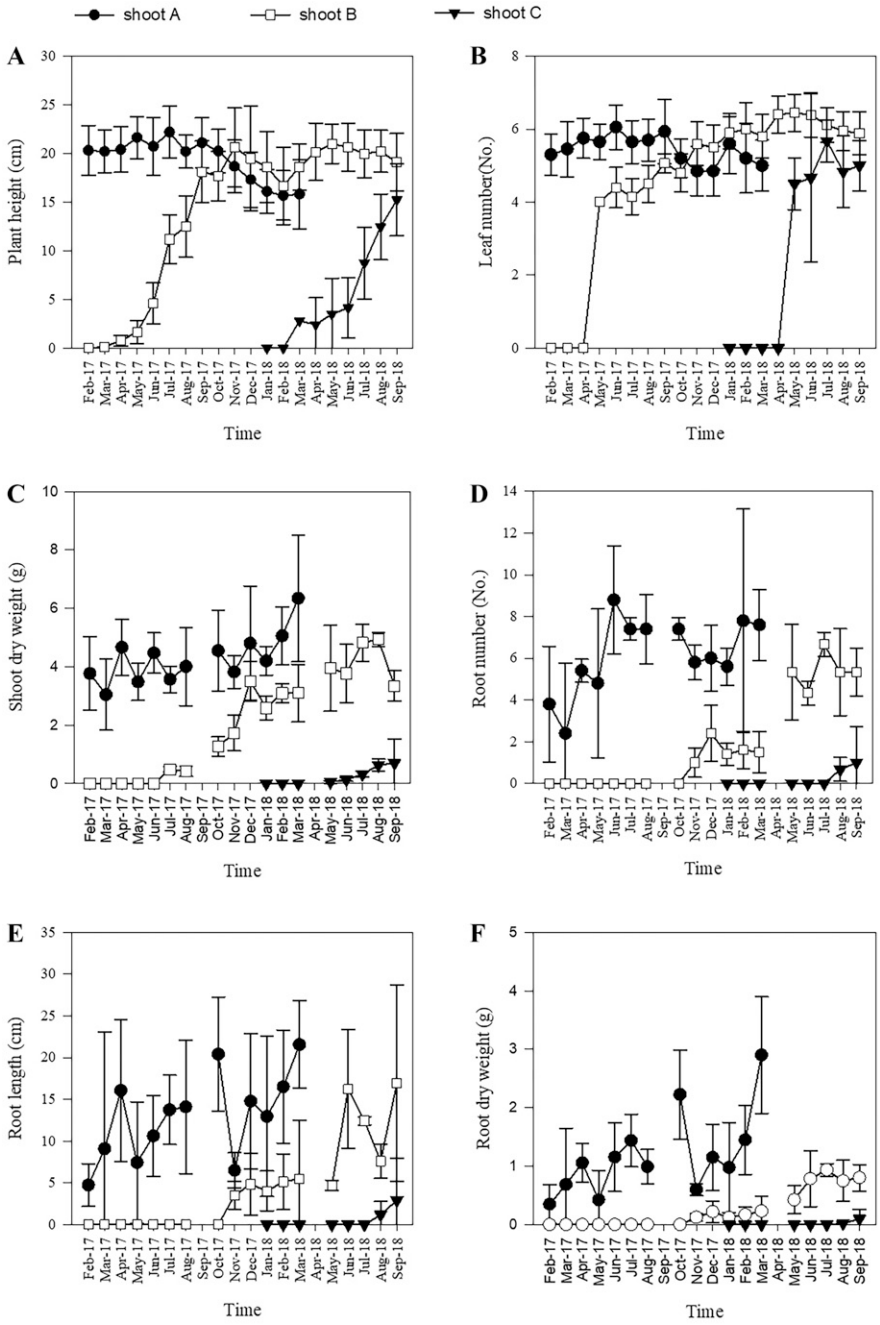

Fig. 5. Dynamic changes of shoot- and root-related traits during two growth cycles of *Paphiopedilum* Clair de Lune 'Edgard Van Belle'. (**A**) Plant height. (**B**) Leaf number. (**C**) Shoot dry weight. (**D**) Root number. (**E**) Root length. (**F**) Root dry weight. A total of 215 plants were analyzed in the first growth cycle, 50 of which were sacrificed for morphological observation; 167 plants were analyzed in the second growth cycle, 30 of which were sacrificed for morphological observation.

the flower bud induction period as potential factors that may affect flower bud induction of *Paphiopedilum*. As proposed by Feng et al. (2021), there are differences in the duration of flower development among *Paphiopedilum* species as a result of the influence of external environmental factors (such as temperature) and the ability to acquire and use resources. It is suggested that *Paphiopedilum* may have the potential for flower bud differentiation throughout the year. However, this process is affected by the timing of plant physiological maturity, the climatic conditions encountered during flower bud induction, and the adaptation of the species to climate change. Consequently, different species exhibit distinct flower bud initiation times. Furthermore, the duration of flower bud development from differentiation to flowering varies among species, resulting in the difference in flowering period.

Growth characteristics and flowering of Paphiopedilum. The relationship between orchid growth characteristics and flower bud differentiation has been studied. Several studies have shown a significant correlation between plant height and flower bud length. For example, in the case of the terminal inflorescence in the *Brassolaeliocattleya* hybrid 'Shinfong Princess', flower bud differentiation began when the new vegetative shoots reached a height of 12 to 18 cm (Wu et al. 2009). However, in the case of *Cattleya labiata* Lindl., there was no significant correlation between flower bud differentiation and pseudobulb height or leaf development phase (Rotor and MacDaniels 1951). Paradiso and De Pascale (2014) proposed that the size of *Phalaenopsis* hybrid plants mainly affects the time required for flower bud induction, rather than the number of days from flower bud differentiation to flowering. This effect is associated with genotype-specific sensitivity, implying that genetic backgrounds and adaptation to the environment both play crucial roles in each species or cultivar.

After two growth cycles, we report regular changes in the growth of *Paphiopedilum* Clair de Lune 'Edgard Van Belle', and the reference growth state when the plants develop to physiological maturity. Notably, the roots of new vegetative shoots begin to develop 5 to 8 months after the shoots have formed. When shoots began flower bud differentiation, the level of root development was still low, indicating that the proportion of nutrients contributed by roots was small in the early phases of shoot development. In sympodial orchids, it has been proved that the back pseudobulbs serve as carbohydrate storage organs to support, in part, the growth of the new vegetative shoot and subsequent flower development. During inflorescence development, the main source of nutrient supply includes the photosynthetic products of the current pseudobulb, followed by the photosynthetic products stored in the back pseudobulb (Lin and Chang 2023; Yong and Hew 1995). Although *Paphiopedilum* does not have obvious pseudobulbs, their leaves have large epidermal cells and a root cortex composed of large parenchymal cells, suggesting that they function as water and nutrient storage organs (Shiao 2000). We demonstrated that the dry weights of the back shoots and their roots continue to increase after flowering. Therefore, we speculated that *Paphiopedilum*, despite the lack of distinct pseudobulb structures, may still have the ability to provide nutrients for the growth of new shoots. However, further experiments are needed to confirm this.

Temperature and flower bud drop. In our study, an average daytime temperature of 29.0 °C and an average nighttime temperature of 26.5 °C caused a high rate (33%) of flower bud drop in *Paphiopedilum* Clair de Lune 'Edgard Van Belle'. The main period of flower bud drop was from July to September, which corresponds to the rapid elongation of the flower stalk until the floral organs mature. During this period, flower buds are constructed rapidly, a large amount of assimilates are consumed, and the plants are exposed to higher temperatures. [From June to August, the hours at more than 30 °C reached 118 to 160 h per month (see Supplemental Fig. 1).] Although high temperatures promote vegetative growth and enhance flower quality, excessively

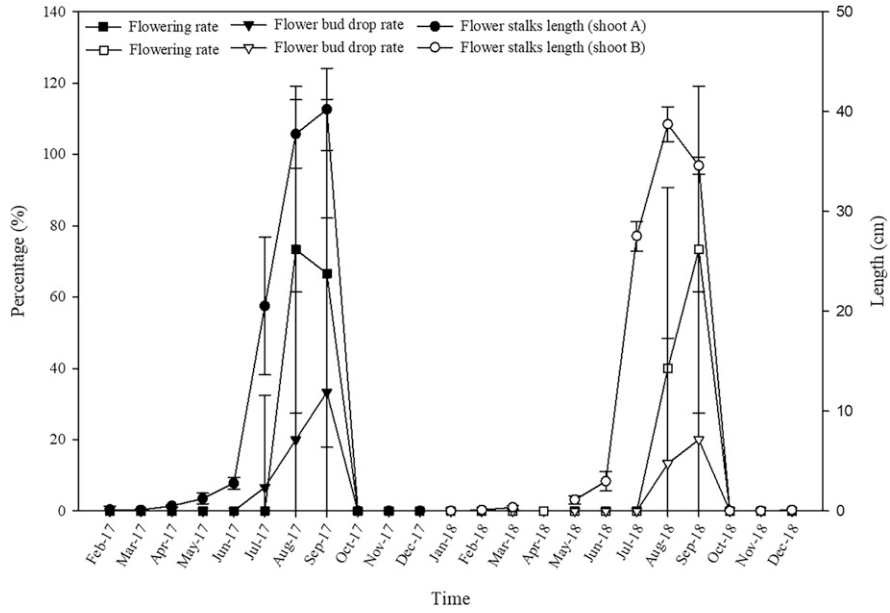

Fig. 6. Flower phenology of *Paphiopedilum* Clair de Lune 'Edgard Van Belle' from Feb 2017 to Dec 2018. The percentage of reproductive stalks with open flowers, the bud drop rate, and the flower stalk length were determined for 215 plants the first year and 212 plants the second year.

high temperatures have been shown to cause flower bud abortion and lower flower quality (van Tongerlo et al. 2021; Zheng et al. 2010). High temperatures reduce the photosynthetic capacity of the plants, lead to a decrease in carbohydrate content (Jeong et al. 2020), and reduce the ability to allocate assimilates to inflorescences (Liu et al. 2013). Furthermore, we also found that stamens were the first floral organ to turn brown during the development of floral organs (Fig. 7D). This is consistent with the observation that male floral organ development is more sensitive than female floral organ development to increased temperature or other abiotic stresses in many higher plants (De Storme and Geelen 2014; Smith and Zhao 2016). The adaptive capacity of a species to environmental temperature is also related to its genetic characteristics. Feng et al. (2022) pointed out that different species of *Paphiopedilum* have distinct leaf structural plasticity, resulting in varying photosynthetic rates and growth rates in response to the same temperature changes. Based on the results of our study, it can be inferred that *Paphiopedilum* Clair de Lune 'Edgard Van Belle' is a cultivar that is relatively sensitive to high temperatures during flower development, leading to flower bud drop. In fact, our tests found that when the flower stalks of *Paphiopedilum* Clair de Lune 'Edgard Van Belle' grew to more than 20 cm, 50% of the flower buds dropped when exposed to a temperature of 35 °C for 4 h a day for more than 2 weeks. When the treatment time at 35 °C was increased to 6 h, 100% of the flower buds dropped within 1 week (data not shown).

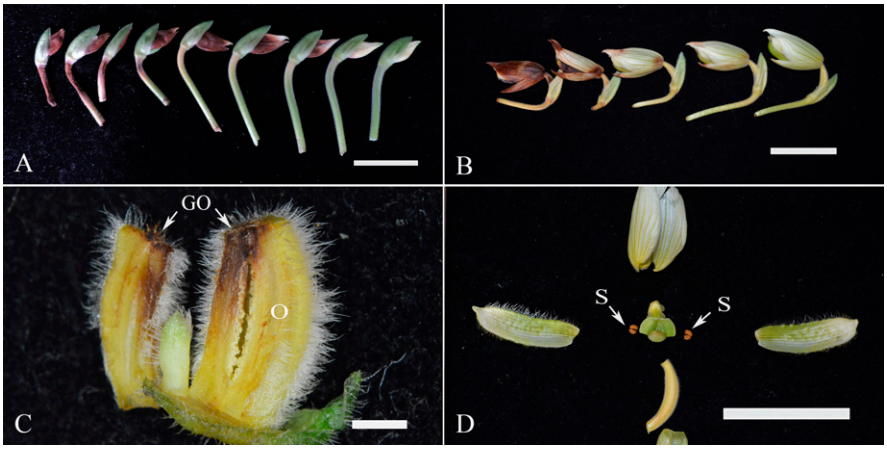

Fig. 7. Morphology of flower bud drop in *Paphiopedilum* Clair de Lune 'Edgard Van Belle'. (**A**) Images of fallen inflorescences in July. Bar = 5 cm. (**B**) Images of fallen inflorescences in August. Bar = 5 cm. (**C**) During rapid flower stalk elongation (Julu), the falling inflorescences turned brown preferentially at the junction of the ovary and gynostemium. Bar = 0.2 cm. (**D**) In the later phase of flower bud development (August), the dropped flower buds were preferentially browned in the stamens. Bar = 5 cm. GO = gynostemium–ovary junction; O = ovary; S = stamen.

Conclusion

In our study, we investigated the growth cycle and plant development characteristics of *Paphiopedilum* Clair de Lune 'Edgard Van Belle' in plants that have been propagated by artificial division for a long time. Nonprimary flowering mature plants and with one or two back shoots are ready for reproductive growth when the average plant height of the new vegetative shoots is more than 20 cm, there are five leaves, and the average shoot dry weight exceeds 3 g. However, due to the root development level of new vegetative shoots is still low at this time, as well as the dry weights of the back shoots and their roots continue to increase after flowering. So, we thought that during the early development of new vegetative shoots and the development of flower buds, shoots from previous generations contribute stored assimilates.

Paphiopedilum species differ in their ability to adapt to environmental changes and vary in the rate at which flower buds develop, resulting in significant differences in flowering time among species, and may be sensitive to changes in ambient temperature during the later phase of flower development. Therefore, we suggest that for the efficient cultivation and flowering control of *Paphiopedilum*, the first issue is to understand the suitable growth conditions of the cultivar to accelerate the maturity of the plant and transition it into the irreversible flower bud differentiation phase. It is also necessary to avoid stress that may occur during flower development (resulting in delayed flower development or flower bud drop), which will help improve flowering quality. For *Paphiopedilum* Clair de Lune 'Edgard Van Belle', controlling the cultivation temperature at 25 to 30 °C should help to accelerate plant maturity and to avoid flower bud drop.

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