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Understanding the Flowering Behavior of Pigeon Orchid (*Dendrobium crumenatum* Swartz)

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ABSTRACT

Inflorescences of pigeon orchid (*Dendrobium crumenatum* Sw.) are comprised of 3 floral buds, each at a different developmental stage. Only one floral bud at the responsive stage could be stimulated to flower at exactly 9 days after the rainfall event (days to anthesis, DTA=9). Floral buds at this stage were characterized as perfect miniature flowers in which microsporocytes were in a pre-meiotic stage. The binucleate pollen grains appeared 3 days prior to anthesis. The conditions on the day of induction, required a decrease of 10°C in temperature from the peak temperature at noon within 1-2 h to trigger events leading to anthesis. Both cooling and a pre-warming treatment before cooling could stimulate anthesis at 19 (DTA=19) and 21 (DTA=21) days after treatment, respectively. Concentrations of 10^{-2} M gibberellic acid (GA_3) and 10^{-1}-10^{-2} M N^6-benzyladinine (BA) applied separately to the inflorescences of grown plants could stimulate anthesis but simultaneous application from all permutations of concentrations of both chemicals failed to do so. *In vitro* clonal propagation from axillary bud culture through callus-derived protocorm like-bodies (PLBs) and plant regeneration was developed through both embryogenesis and organogenesis. *In vitro* floral organs were induced from the 4-week-old PLB-derived plantlets while subsequent floral organogenesis and anthesis were dependent on the conditions of the rainfall event only. The *in vitro* induced plants flowered within 8-12 months as compared to 5-7 years for natural plants. This system shortened the juvenile period of this orchid. This knowledge is very useful and critical for many aspects of future flowering research.

Keywords: anthesis, exogenous application, histology, *in vitro* floral organ induction, plant regeneration, rainfall event
Abbreviations: BA, N^6-benzyladenine; **CW**, coconut water; **DTA**, days to anthesis; **GA_3**, gibberellic acid; **KC**, Knudson C medium; **PGR**, plant growth regulator; **PLB**, protocorm-like body; **VW**, Vacin and Went medium

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INTRODUCTION

The flowering process is essential for the production of edible food crops, and horticultural and floricultural crops. A detailed understanding of what controls flower development is extremely important for devising means to efficiently regulate flower formation (Yanofsky 1995; Weigel 1998), for instance, in manipulating reproduction for plant improvement (Goh 1992) and in developing new ornamenttal varieties. However, there are still knowledge gaps that need to be bridged for an in-depth understanding of the basic mechanisms controlling flowering. In particular, the physiological and molecular bases of floral induction and morphogenesis have remained a mystery (Aukerman and Amasino 1998; Hempel *et al*. 2000; Datta and Das 2002).

Although the understanding of what might control flowering has changed during the last 30 years because of the development of new molecular biological techniques there are still many questions that need to be answered to understand the complex process of flowering. Therefore, classical knowledge of flowering has to be linked to these newer approaches now available to analyze the flowering process (Yu and Goh 2001). The application of two new approaches is particularly significant, i.e. that of *in vitro* flowering and the molecular analysis of flowering control (Coen and Meyerowitz 1991; Yu and Goh 2000; Yu and Goh 2001). The *in vitro* flowering system will be extremely useful for studying the various control mechanisms in the flowering processes, i.e. floral initiation, floral organ development and floral senescence (Goh 1992), in particular when pollination and fer-

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Invited Review

tilization *in vivo* are difficult. In addition, any lengthy period of vegetative growth normally required before flowering could be significantly shortened. This system combined with the newer molecular techniques could be the ultimate tool for initiating genetic manipulation of the flowering process (Woodson 1991). As a result of these advances in technology, the present understanding indicates that the flowering events of higher plants involves the switching on of a cascade of genes, most requiring the MADs box transcription activator protein (Levy and Dean 1998; Datta and Das 2002). At the molecular level, genes that act to control the initiation and development of flowers have recently been isolated (Coen and Meyerowitz 1991; Yu and Goh 2001). The challenge, then, will be to interpret the function of such genes and use the information for the beneficial manipulation of the flowering process (Thomas 1993). Rapid advances in the understanding of flower development and molecular genetics are currently under way as part of the Floral Genome Project (http://floralgenome.org/). The project will generate a comparative data set of expression pattern for a large number of genes involved in flower development across diverse angiosperms besides testing standard hypotheses regarding the origin of the floral developmental program (Eckardt 2002). At present, researchers from this project at Penn State University with international team of collaborators have proposed an answer to Charles Darwin's "abdominable mystery" (Cui *et al*. 2006; http://biologynews.net/archives/2006/05/11/mass).

Orchids can be found in all parts of the world but their physiological mechanisms of flowering are different from other flowers due to their diverse phylogenetic origins and wide range of ecological habitats (Goh *et al*. 1982; Goh and Arditti 1985). They exhibit specialized morphological, anatomical and physiological characteristics especially in their reproductive biology (Yu and Goh 2001). One of the tropical orchids, *Dendrobium crumenatum* Sw. may be propagated not only as a dwarf-plant but also for fragrance extraction. This orchid species is typical of Malayan orchids and has many uncommon features of flowering, for instance, gregarious flowering (Holttum 1964; Bernier *et al*. 1981a, 1981b; Went 1990; Bernier *et al*. 1993). Flowers bloom exactly 9 days after a rainfall event. All plants in a certain area start to flower at the same time and are at the same stage of floral development throughout the year, but the blooms last for only a full calendar day (Holttum 1964; Goh *et al*. 1982). It is assumed that the time required to complete final floral development is constant in all individuals and development can be predicted to take place at a specific time (Endress 1994). There are many reasons to carry out research on this orchid, for example, floral stages are synchronized and sufficient for group analysis, the floral process is strictly controlled by temperature, and flowers show a consistent rhythm of opening and closing at the same hour of the day. It is of interest to many physiologists, developmental biologists, molecular geneticists, and plant breeders so it is important to conduct research on its flowering behavior (Bernier *et al*. 1981a; Goh and Arditti 1985). In addition, it has been used as an ideal plant for use as a model for molecular research into the physiology of flowering and has been widely studied as an example of flower induction (Goh *et al*. 1982; Went 1990; Goh 1992; Bernier *et al*. 1993; Yu and Goh 2001). Moreover, analyses of comparative genomics can both promote transfer of knowledge from model to non-model systems and inform understanding of conserved processes in model species (Leebens-Mack *et al*. 2006). This review contributes to understanding the general characteristics of the flowering of *D. crumenatum* and explain why flowering behaves in the manner described above.

CLASSIFICATION AND LOCALITY

Recent taxonomy of the orchid family

The orchid family, Orchidaceae, is one of the largest families of flowering plants. Based on their complexity of floral architecture and fertilization mechanisms, this family is regarded as evolutionarily advanced. Classification is still an ongoing issue because about 150 species and even new genera are being discovered each year. In addition the taxonomy of this family is in constant flux, as DNA studies provide new information. The subdivision of the family has proved difficult and classification is diverse. Changes in taxonomy of orchids are so frequent that the following taxonomy still follows largely the system of Robert Louis Dressler. This classification, published in the book *The Orchids: Natural History and Classification*, is widely accepted by botanists and growers at the moment. The initial scheme of 1981 has been modified in 1986, twice in 1990 and then again in 1993 (http//en.wikipedia.org/wiki/Taxonomy_of_the_Orchid_family). The family is subdivided into several subfamilies and then into tribes, subtribes, alliances and then genera. There are 5 subfamilies (1, Apostasioideae; 2: Cypripedioideae; 3: Orchidoideae, including Spiranthoideae; 4: Epidendroideae, including higher Epidendroideae (formerly Vandoideae); 5: Vanilloideae), 22 tribes, 70 subtribes, about 850 genera and about 20,000 species. The subfamily Vanilloideae is a branch at the basal dichotomy of the monandrous orchids and it is a sister to the subfamily Epidendroideae + subfamily Orchidoideae.

Classification of pigeon orchid (*Dendrobium crumenatum* Sw.)

Common names: dove orchid, pigeon orchid, sparrow orchid; Thai name: Wai Ta-Moy; distribution: South China, Indochina, Burma, Malay Penisula, the Phillippines to South-east Asia, Indonesia to New Guinea.

Dendrobium is a monandrous orchid with monosymmetrical flowers. It has been placed in subfamily 4 (Epidendroideae), the largest subfamily comprising more than 10,000 species in about 90 to 100 genera (Endress 1994). The pigeon orchid, *Dendrobium crumenatum* Sw., is a tropical epiphytic plant 40-100 cm in size and usually grows on old trees in the open country. The flower (3.5-4.5 cm) is produced from the chaff bract-clusters along the upper part of stalk-like plant body portions. A flower has three sepals, two petals, a lip or labellum (median petal), a gynoecium with a tricarpellate and inferior ovary. The stigmas, styles and stamens are congenitally fused into a single structure called the column or gynostamium. The fused basal part of the lip and the lateral sepals (sometime also the petals), appear as a ventral extension of the column carrying the lip and forming a column foot. The column foot and sepals may form a mentum or chin-like projection. As the bud starts to open (or just prior to that), the pedicel twists 180 degrees in a process called resupination. The pollen grains are produced in masses known as pollinia. The fruit deve-

Fig. 1 Opened flower of Wai Ta-Moy orchid (*D. crumenatum*) shows pure glittering white and a bright yellow disc on the lip.

lops into a capsule. The flower has a strong fragrance, is a pure glittering white with the lip having a bright yellow disc (**Fig. 1**).

REPRODUCTIVE DEVELOPMENT

The design of the plant body is established during embryogenesis when the undifferentiated meristematic regions of root and shoot are set aside followed by plant development that occurs postembryogenically through the production of an organ primordium at the shoot apical meristem (SAM). The SAM gives rise initially to vegetative organs and sometimes makes the transition from vegetative to reproductive development (Aukerman and Amasino 1998). SAM changes are controlled by environmental and endogenous signals besides genes and processes that are involved in reproductive development. A series of genes is involved in the conversion of the SAM to an inflorescence meristem (IM) and floral meristem (FM) (Datta and Das 2002). In the first case, the SAM is converted into an IM and this IM produces a series of FMs. Each FM ultimately gives rise to a single flower with floral parts. In the second case, SAM or the vegetative meristem (VM) is transformed into an IM which then generates the FM. However, this IM neither produces floral organs directly nor generates leaves. It produces an FM with bracts or sometimes a mixture of FMs and more IMs in the axils of the bracts followed by the floral organs which are produced in succession by the activity of the FM. Floral development of *D. crumenatum* is in accordance with that of other Epidendroideae and Vandoideae described by Kurzweil (1987). At the early stage of floral development (**Fig. 2**), the labellum is pointed upward (in the non-resupinate position). The lateral sepals (S2, S3) are visible on the adaxial side of the mound followed by the lip (L). The abaxial side is divided and elevated into the lateral petal primordia (P1 and P2) followed by the median sepal primordium (S1). The anther primordium (A1) is initiated from the abaxial ridge above the S1 (episepalous position). While the domed anther grows in an upright position and becomes flattened to a broad tongue-like structure, all sepals (S1-S3) are positioned higher to cover the young floral bud. Late development, i.e. the gynostamium development, the anther and pollinia development has been discussed in the first series devoted to the affinities of the Epidedroideae and Vandoideae described by Kurzweil (1987). The labellum is in a downward (or resupinate position) when the flower opens resulting in a 180° twisting of the pedicel (Goh *et al*. 1982).

GROWTH AND FACTORS AFFECTING FLOWERING

In general the two important factors that induce flowering are low temperature and light. Pigeon orchid has been shown to respond to low temperature. The phenomenon of *D. crumenatum* flowering, i.e. its detailed classical and neo-biotechnological characteristics, are now being investigated. A marked decrease in temperature is required for this orchid to flower. However, the conditions necessary for flower bud initiation might differ from those for bud growth and development. The availability of nutrients for the developing flowers and during flowering is also important. Nutrients and water from the pseudobulb and leaves can be transportted into the developing flower (Goh *et al*. 1982; Meesawat and Kanchanopoom, unpublished data). In some plant species, nutrition availability, i.e. nitrogen, is closely bound to the flowering response unlike the majority of plants.

Flower miniatures at the responsive stage

The mature plants produce inflorescences at intervals, at the nodes of the inflorescence axis. Each short inflorescence consists of two to three floral buds, each covered by bracteal leaves, at different developmental stages. All floral buds in an inflorescence remain dormant and wait for a rainfall event to stimulate further floral development. This orchid will not flower until the floral bud reaches the stage of readiness to respond, termed the responsive stage. Only the 1st floral buds at the responsive stage flower completely, exactly 9 days after the rainfall event (day to anthesis, DTA=9). While the 1st floral bud opens, the 2nd and the 3rd floral buds are developing and subsequently turn into the 1st and the 2nd floral buds, respectively. The time of subsequent development from the 2nd to the 3rd buds is approximately 25-30 days after the rainfall event or natural stimulus (unpublished data). This means that the next flowering will appear when the floral bud is in the responsive stage combined with the next rainfall event. The flowering cycle occurs until there are no floral buds on the inflorescence. The events that occur in this subsequent flowering are shown as a diagram (**Fig. 3**). The 1st floral bud at the responsive stage, the stage at which the floral bud responds to the rainfall event, ap-

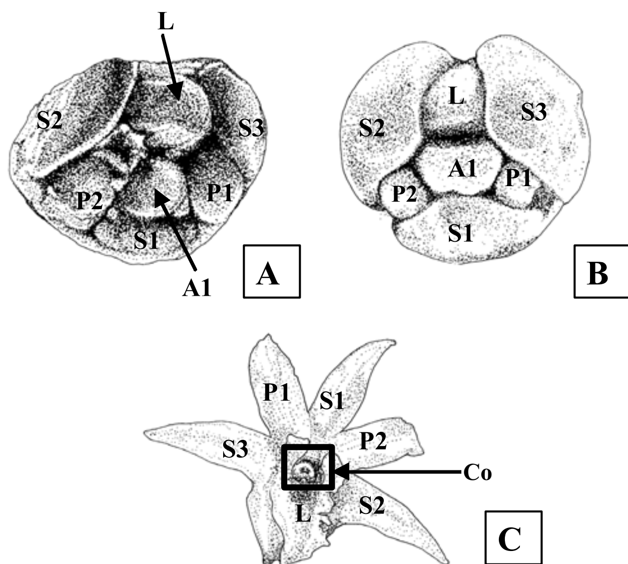


Fig. 2 Drawing showing the initial differentiation of flower primordia of *D. crumenatum* at the early stage in non-resupinate position (A, B) and the twisting of the pedicel (180°) in resupinate position at flower opening (C). A1, anther primordium; Co, column/gynostamium; L, lip/ labellum; P1-P2, petals; S1-S3, sepals.

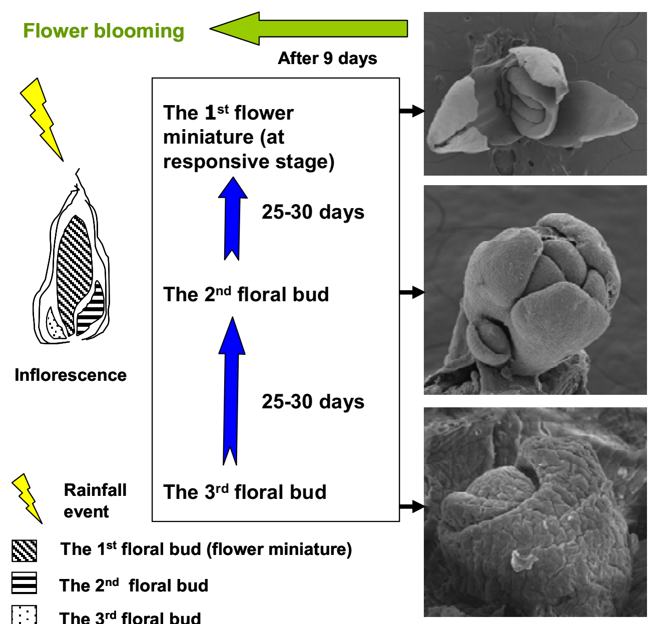


Fig. 3 Possible events during the natural flowering of *D. crumenatum*. The first floral bud of *D. crumenatum* exhibits the miniature flower completed with all floral parts at the responsive stage waiting for the rainfall event.

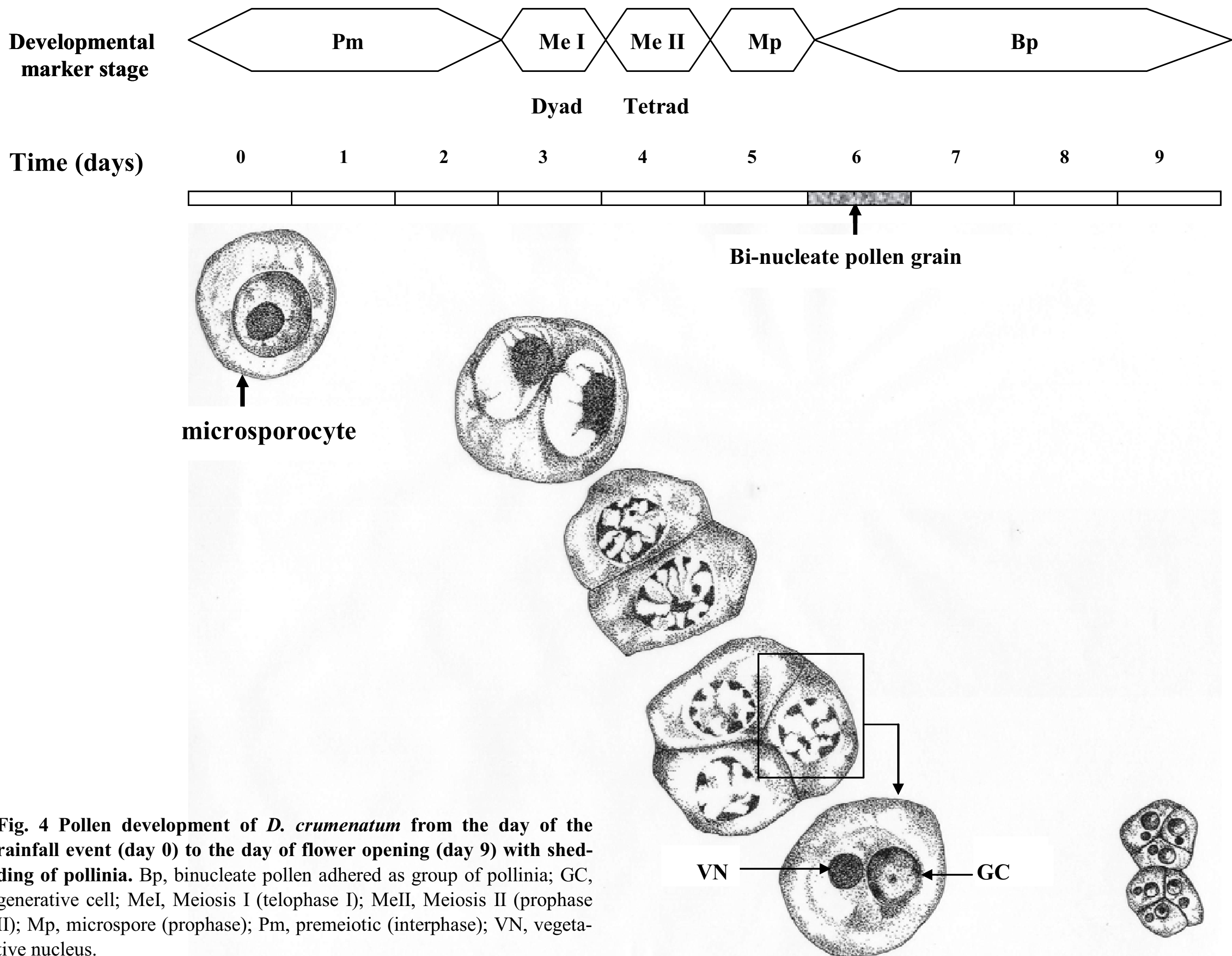


Fig. 4 Pollen development of *D. crumenatum* from the day of the rainfall event (day 0) to the day of flower opening (day 9) with shedding of pollinia. Bp, binucleate pollen adhered as group of pollinia; GC, generative cell; MeI, Meiosis I (telophase I); MeII, Meiosis II (prophase II); Mp, microspore (prophase); Pm, premeiotic (interphase); VN, vegetative nucleus.

pears as a miniature version of the completed flower with all floral parts and the microsporocytes (2n) still being at a pre-meiotic stage (**Fig. 4**). These conditions are critical and necessary for normal flowering of *D. crumentaum* orchids. The 2-celled pollen grain, a large vegetative nucleus (VN) and a small generative cell (GC), appear 6 days after the rainfall event or 3 days before anthesis. A group of pollen grains, known as pollinia, adhere to and remain together until anthesis. Pollen development of this orchid is synchronous.

Marked decrease in temperature and low temperature

Anthesis in *D. crumenatum* is stimulated by rainfall and the associated cooling. After rain, the temperature will drop from the peak temperature at noon by between 7.66-9.69°C, within 1-2 h (**Fig. 5A**). A gradual decrease of temperature on a cool day or cool night has no effect (**Fig. 5B**). Test results from cold-treated plants support the importance of a speedy chilling although only a few flowers might open. Plants kept in the dark, at a temperature of 8 ± 2°C (CR) for between 1 and 5 h, could break dormancy of *D. crumenatum* flowers after being placed in natural conditions (**Fig. 6**, Meesawat 2006). Unfortunately, in this experiment it took 19 days for anthesis (DTA=19), i.e. a lot longer than in nature (DTA=9). A warming-up period to 37°C for 1 h before the cold treatment (HCR) for 1 and 5 h induced flowering after 21 days (DTA=21). Most floral buds from both CR and HCR experiments that were developing after 3 h had aborted by the 3rd day after treatment. Damage caused by chilling after longer periods of cooling is generally manifested by yellowish-dry leaves and wilted shoots.

Exogenous application of cytokinin and gibberellin

Cytokinin, BA, gibberellin, and GA_3 play an important role in the control of *D. crumenatum* flowering. The application of BA at 10^{-1} and 10^{-2} M stimulated flowers with 9 and 11 DTAs, respectively (Meesawat 2006). A DTA of 9 was similar to that under natural conditions. Six individual plants treated with 10^{-1} M BA developed floral buds (58.33%) some of which flowered (14.29%) (**Fig. 7**). Applying 10^{-2} M GA_3 resulted in flowering with a DTA of 10. The percentage of flowering responses from the application of 10^{-1}, 10^{-2} M BA and 10^{-2} M GA_3 was 8.33%, 9.09% and 12.50%, respectively. However, simultaneous application of all the permutations of concentrations of a combination of GA_3 and BA failed to induce any flowering. Cytokinins can induce flowering in sympodial orchids, i.e. *Dendrobium* hybrids (Goh *et al*. 1982; Goh 1992). Benzyladenine (BA) caused an increase in the length of the inflorescence and the numbers of flowers in *Dendrobium* cv. 'Louisae' (Goh *et al*. 1982). Gibberellins induced flowering in *Bletilla striala*, *Cymbidium* and *Cattleya* hybrids (Goh *et al*. 1982). Goh *et al*. (1982) revealed that gibberellic acid (GA_3) brought about an increase in flower size and accelerated the flowering in some *Cymbidium* hybrids. The cytokinin effect was also efficiently enhanced by the simultaneous application of GAs (Goh 1992).

IN VITRO BIOTECHNOLOGY

Tissue culture techniques have advanced rapidly in recent years and have become an acceptable technique for clonal propagation. *In vitro* plant regeneration is the basis for various further studies, i.e. *in vitro* flowering systems (Chang and Hsing 1980; reviewed in Teixeira da Silva

Fig. 5 Typical patterns of temperature variation throughout the day on induction days, or the day of rainfall event (A), and on non-induction days (B).

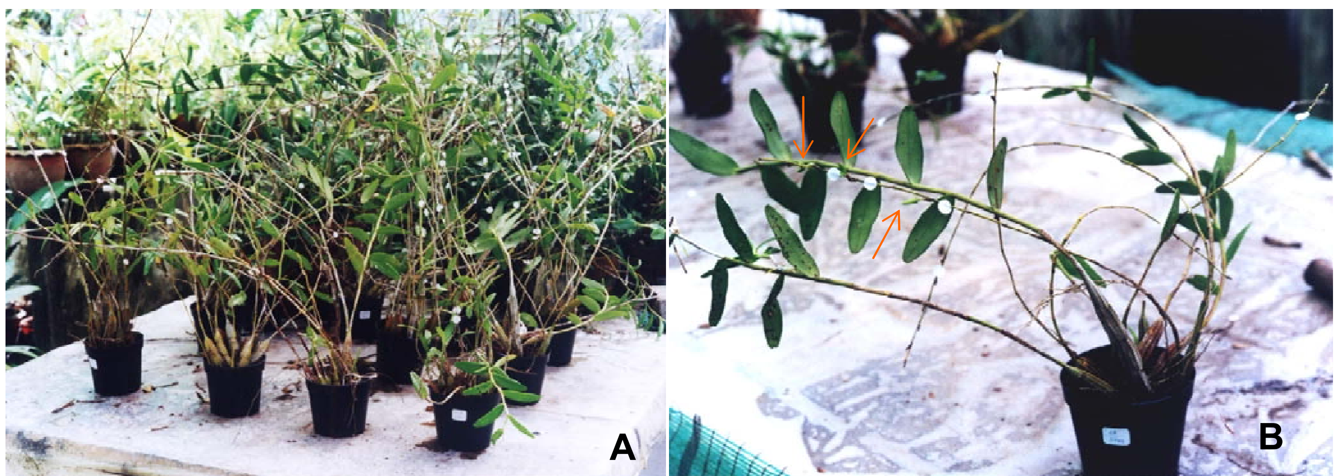


Fig. 6 Cold-treated and pre-warm before cold treated experiments being placed in uncontrolled conditions of greenhouse (**A**). Plants from 5 h cold-treated experiment showing the 3 developing flower buds (arrows). Photographs were taken on the day of treatment (**A**) and 2 days before flower opening (**B**) or 17 days after given treatment (DTA=19).

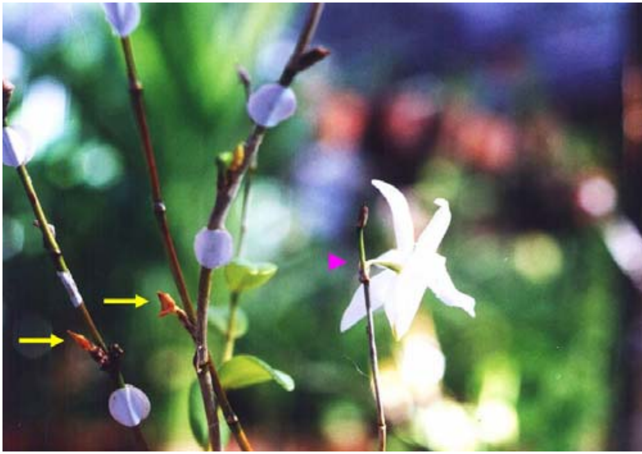


Fig. 7 Flowering response of 10^{-1} M BA treated plants grown in a natural greenhouse. All six individual plants of 10^{-1} M BA treatment provide developing floral buds (58.33%). However, some developing flower miniatures had gradually died since the 5th day (arrows) while the others could further develop to flower (arrow head) 9 days after the first given treatment (DTA=9). Photograph was taken 9 days after hormone treatment.

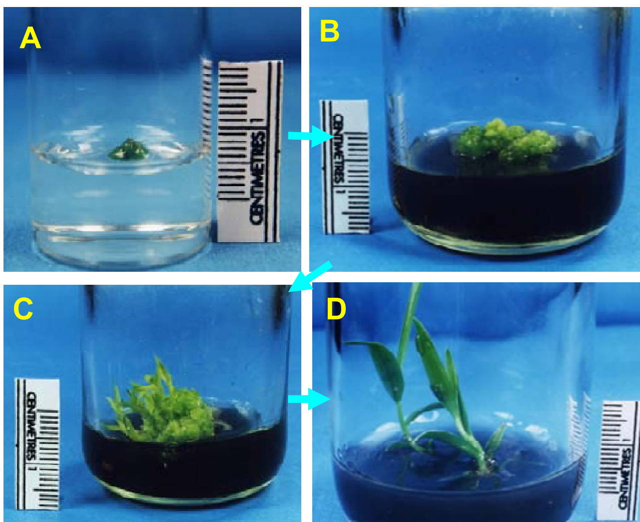
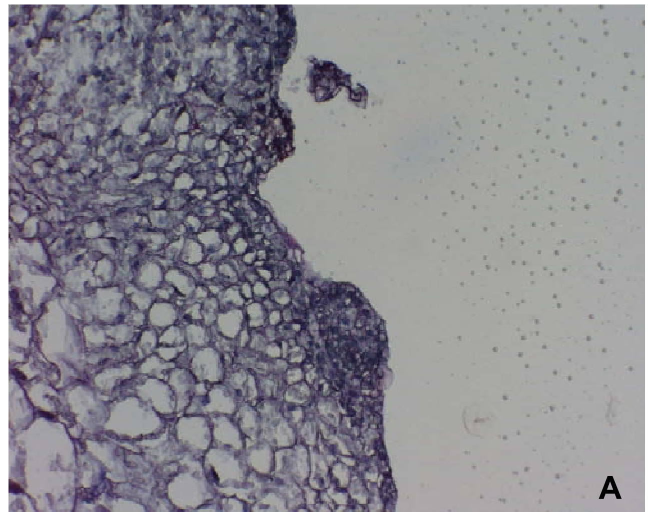


Fig. 8 Plantlet formation *in vitro* in *D. crumenatum*. (**A**) Bud culture; (**B**) callus formation; (**C**) PLB and shoot formation; (**D**) plantlet development. Figure reprinted from **Meesawat and Kanchanapoom** (2002) *Thammasart International Journal of Science and Technology* **7**, 9-17, with kind permission from the editor and publisher.



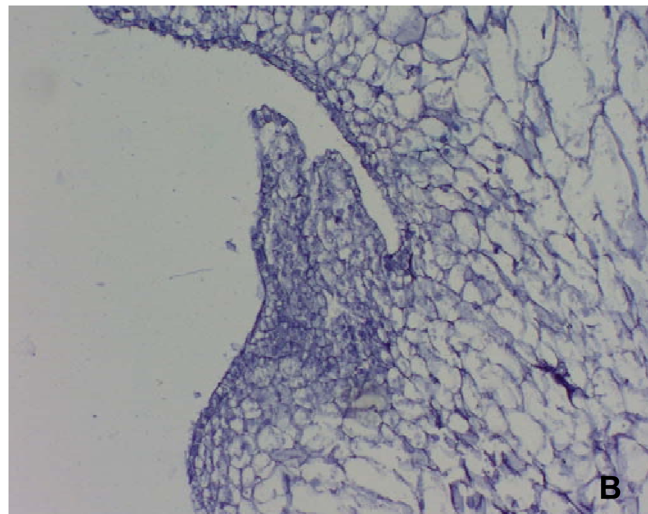


Fig. 9 Dense meristematic tissues showing the early stage of somatic embryogenesis (**A**) and shoot meristem with leafy organ like structures indicating organogenesis development (**B**).

2006) and genetic modification studies (Woodson 1991). Uniform plantlets of *D. crumenatum* are easily produced. Apical buds were cultured on Vacin and Went (VW, Vacin and Went 1949) solid medium supplemented with only 20-30 g.l^{-1} sucrose before being transferred to VW medium supplemented with 1 mg.l^{-1} BA, 0.1 mg.l^{-1} NAA, 2 g.l^{-1} peptone, 20 g.l^{-1} sucrose, 2 g.l^{-1} activated charcoal (AC) and 2 g.l^{-1} Gelrite and callus was induced within one month. The callus was then proliferated on the same medium (Meesawat and Kanchanapoom 2002). The green or yellowish callus formed proliferating nodular compact structures (NCSs), and developed into protocorm like-bodies (PLBs) and differentiated into regenerated shoots by the 4th, 10th and 16th week, respectively (**Fig. 8**). PLBs were formed on modified VW medium with a combination of 1 mg.l^{-1} BA, 0.1 mg.l^{-1} NAA supplemented with 10% coconut water (CW) or modified VW medium supplemented with only 10% CW. The most vigorous shoots were obtained from a hormone-free VW medium with 2 g.l^{-1} peptone and 7.2 g.l^{-1} agar. The regenerated shoots with 2-3 leaves were detached from shoot clumps and transferred to VW medium without hormone addition for rooting. They were separated and transferred to fresh media. All media were supplemented with 20 g.l^{-1} sucrose, 2 g.l^{-1} AC and the pH was adjusted to 5.3. After that, the detached plantlets having 2 expanded leaves and 2-3 roots were surrounded by moist coconut peat before being fitted into the mini-pots and transplanted to greenhouse conditions. Histological observations revealed that the regeneration pathway was developed through two morphogenic routes: embryogenesis and organogenesis (**Fig. 9**). However, embryogenesis occurred more than did the organogenesis (Meesawat and Kanchanapoom 2002).

An *in vitro* flowering system is a new route and a valuable tool for flowering research. This provides a system to study specific aspects of flowering, to shorten the breeding period of plant species and to analyze gene expression during floral development (Goh 1992; Yu and Goh 2000; Yu and Goh 2001; reviewed in Teixeira da Silva 2006). It could be applied to regulate or promote flowering in relatively short periods. This system offers potential value in breeding programs by shortening the time for explants to initiate the first floral bud (Kerbauy 1984; Duan and Yazawa 1994; Wang and Zu 1996). This *in vitro* flowering system has been studied in many plants (reviewed extensively by Taylor and van Staden 2006), and many orchid species and their hybrids (Kerbauy 1984; Duan and Yazawa 1994, 1995; Kostenyuk *et al*. 1999; Sim *et al*. 2007). *In vitro* floral bud formation and outdoor flowering of *D. crumenatum* orchid has also been reported (Meesawat 2006; Meesawat and Kanchanapoom 2006).

To illustrate this, the callus-derived PLBs were induced to undergo formation of mini-shoots in modified liquid Knudson C (KC) medium (Knudson 1946) supplemented with 1 mg.l^{-1} BA, 20 g.l^{-1} sucrose, 15% CW. They were placed on a rotary shaker with an agitation speed of 12 rpm

Fig. 10 Young plants grown in a greenhouse from an *in vitro* floral induction source exhibiting elongating apices of the reproductive parts (arrows) that would subsequently give rise to flowers after the repeated rainfall event.

under a 16 h photoperiod with an illumination of 20 µmol $m^{-2}s^{-1}$ photosynthetic photon flux density provided by a Grolux lamp. These cultures were maintained at 24±2°C. The mini-shoots (0.3-0.5 cm long) were treated in the same liquid KC medium containing 5 $mg.l^{-1}$ BA, a higher concentration of BA than in medium for mini-shoot formation, to induce floral buds and floral organogenesis *in vitro*. The monthly sub-culture was done until explants with emerging shoot apices (1-2 cm long with 2 leaves) were detected and then transferred to the solid KC medium supplemented with 1 $mg.l^{-1}$ BA overlaid with liquid KC medium supplemented with 5 $mg.l^{-1}$ BA. Sepal and petal primordia were initiated at weeks 25-30. The explants must be transplanted to solid medium covered by liquid medium before they produce malformed floral buds (at the 32nd week). Unfortunately, no sign of column initiation was detected in treated liquid medium. The explants with induced floral buds were unable to further develop into flowers opening *in vitro* unless they were both transplanted to natural greenhouse conditions and received the rainfall event (**Fig. 10**). It was interesting that all plants synchronously flowered 9 days after the rain-fall event. It took about 8-12 months from the beginning of the bud culture to obtain flowers (**Fig. 11**).

Classic experiments (artificial stimulation)

-BA, GA application (DTA= 9,10,11)

-Cold treatment (DTA= 19, 21)

Flower blooming

Natural stimulation

Rainfall event (DTA= 9)

(difference in temperature drop ~10 °C)

Grown Plants in natural conditions

9 days later

1-2 m

***In vitro* plant regeneration**

Outdoor flowering

transplant

Bud culture (SM)

Plantlets

1 m

1-2 m

Callus initiation (SM)

SM covered by LM

1 m

Long-term culture 7-8 m

Callus proliferation (SM)

(monthly subculture)

Elongating shoots (LM)

3-4 m

***In vitro* floral bud induction**

1.5 m

PLBs (SM)

1 m

Mini shoots (LM)

(monthly subculture)

1 m

Mini shoots (SM) 2-3 leaves

Malformed floral buds

transplant

Plantlets in mini pots

Fig. 11 Schematic illustrating the protocol for *D. crumenatum* flowering in a short period of time. DTA, days to anthesis; LM, liquid medium; SM, solid medium; m, month.

GENETIC STUDIES

Manipulating the molecular biology that controls the flowering process is critical for controlling the flowering of many floricultural crops. It has also contributed to our basic understanding of flowering physiology. While *Pharbitis nil* is a critical short-day plant that has served as a model for the photoperiodic induction of flowering (Gao *et al*. 2006; Zielinska *et al*. 2006), *D. crumenatum* can be used as a model for a plant requiring cold induction. Much effort has been expended on identifying and characterizing the genes that play a role in the transition from vegetative to reproductive development. For the typical angiosperm flower, 4 different floral organs, namely; sepals, petals, stamens and pistils are initiated sequentially in the flanks of the FM to form the whorl of calyx, corolla, androecium and gynoecium, respectively. Each whorl is determined by a unique combination of 3 organ specific genes or ABC-class of genes (Datta and Das 2002). This classical ABC model was originally postulated by Coen and Meyerowitz (Coen and Meyerowitz 1991; Weigel and Meyerowitz 1994). It describes 3 classes (each consisting of one or more genes) of genes that are responsible for the production of a specific floral organ. Activity or function of the type A gene alone specifies sepals whereas the activity of both type A and B genes is required for petals. Stamens are formed by the activity of both B and C genes while carpels need the activity of the C gene alone. In this last decade, the ABCDE model has been proposed to explain flower development in various plant groups (see several reviews in Teixeira da Silva 2006). This revised ABCDE model is an extension on the ABC model. It describes 5 classes of gene functions. Two classes with new functions, D and E, have been added. The function of D and E determine ovule development and development of all the floral organs, respectively. This revised model is also supported by the experimental data in dicotyledonous species, i.e. *Arabidopsis* and *Petunia*. Briefly, the A function is required for sepals, ABE together for petals, BCE for stamens, CE for carpels and D most likely together with C and E for ovule determination. Due to the modifications of the perianth organs and the fusion of reproductive organs in orchid flowers, they are another species useful for studying the genetic basis of flower development. In the *Dendrobium* orchid, there has been some success at forming libraries of cDNA from lateral organs at different developmental stages (Goh 1992). In addition, candidates for the A, B, C, D and E function genes including *AP2*-, *PI/GLO*-, *AP3/DEF*-, *AG*- and *SEP*-like genes have been isolated from *D. crumenatum*. The B and C function genes have been characterized. For instance, the B function, by the members of *AP3/DEF*- and *PI/GLO*- lineages, is necessary for the petal and stamen formations. It was reported that there is partial conservation in the B and C function genes between *Arabidopsis* and orchid. Otherwise gene duplication might have led to the divergence in gene expression and regulation, resulting in the unique floral ontogeny in orchids (Xu *et al*. 2006).

FUTURE PROSPECTIVES

Some detailed information on the flowering of *D. crumenatum* orchid is available and needs to be experimentally confirmed. The fusion of classical knowledge and modern genetics will be very promising for orchidology. This orchid has advantages for providing some special knowledge on the developmental of a complex flowering system, for instance floral organ development, pollen development with respect to regulation of microsporogenesis and development in programmed cell death. The modern techniques of both *in vitro* flowering and molecular studies will make a significant contribution to understanding the physiology of orchid flowering. The *in vitro* studies could promote production of floral buds in PLBs and subsequently *in vivo* anthesis in a short period. Moreover, plant growth and development including floral development will be studied using molecular techniques of gene cloning, expression and characterization of genes. cDNA libraries have been prepared from the floral organs at different developmental stages and homeotic homologues controlling flowering and the genes involved in floral development could be successfully cloned. Modified flower crops such as varieties with modified colors and with enhanced vase life, can be expected in the near future.

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