

# 1. INTRODUCTION

## 1.1 Introduction

Flowering is essential and critical to the production of edible food crops, and horticultural and floricultural crops. The morphogenesis of vegetative meristem into reproductive meristem has attracted scientists for a long time. However, there are still knowledge gaps which need to be bridged for an in-depth understanding of the basic mechanisms controlling flowering. Particularly, the physiological basis of floral induction and morphogenesis as well as the molecular basis of floral induction has remained a mystery (Aukerman and Amasino, 1998; Hempel *et al.*, 2000; Datta and Das, 2002). From previous researches, however, the timing of the flowering in many species has been made progress and many aspects of the flowering process are being elucidated in some crops. Undoubtedly, in the future more advanced understanding will open up new vistas of science and provide us a better capability to manipulate the flowering processes for plant improvement (Goh, 1992). Because the understanding and controlling of how flowers develop is of prime importance in devising efficient means to regulate floral form (Weigel, 1998; Yanofsky, 1995), manipulating reproduction can have a significant impact on the yield of some important species, whereas altering floral structure is of wide interest to scientists in developing new ornamental varieties.

Orchidaceae is one of the largest families of flowering plants. Its members can be found in all parts of the world. The flowers vary greatly in size, shape and color.

These attributes together with their long shelf life are the reason for their being the horticulturally important. In several tropical countries, orchids have become the major flower crops for export. The orchids which are commercially important as being cut flowers belong to relatively few genera such as *Cattleya*, *Cymbidium*, *Dendrobium*, *Oncidium*, *Phalaenopsis*, *Vanda*, etc., and their hybrids. Despite the major economic importance of orchid flowers, only few studies on their flower initiation and development have been undertaken until recently. The physiological mechanisms of flowering of these orchids are different due to their diverse phylogenetically origins (Goh *et al.*, 1982; Goh and Arditti, 1985). A potentially significant application of flowering research, for instance, the mechanisms controlling flowering, could be studied with an *in vitro* experimental system. With this system, the breeding cycle as well as the long period of growth will be significantly shortened. This experimental system will also help to overcome the problem of isolation distance. The long period of flowering and pod formation under field conditions are also considerably shortened. *In vitro* flowering method may be useful when pollination and fertilization *in vivo* are difficult. In the immediate future, it will be necessary to study the flowering processes from two new approaches: *in vitro* flowering and molecular analysis of flowering control (Coen and Meyerowitz, 1991; Yu and Goh, 2000a, Yu and Goh, 2001). For instance, the *in vitro* flowering system will be extremely useful for studying the various control mechanisms in the flowering processes including floral initiation, floral organ development and floral senescence (Goh, 1992). Using this system combined with the molecular techniques will be the ultimate tools of genetic manipulation of flowering (Woodson, 1991). These studies will contribute greatly to our understanding of physiology of flowering in orchids.

One of the tropical orchids, *Dendrobium crumenatum* Sw. may not only be developed as the export dwarf-plants and for fragrance extraction but is also important in flowering research (Bernier *et al.*, 1981; Goh and Arditti, 1985). Patterson and Reid (1990) revealed that plant species whose natural habitats lie in the equatorial region were sensitive to chilling. A case in point being the flower development and anthesis in *D. crumenatum*; flowering induction was initiated by rainfall and associated cooling. However, the flower buds of this orchid remained dormant at the responsive stage, and waited for a stimulus to induce full development. Thus, this valuable orchid not only could be a physiological model of plant that require a low temperature for flowering but also promised to be a useful model for floral development at the molecular level (Goh, 1992; Yu and Goh, 2001). It is in this context that research on the proper stimulus and its limitations is of general interest. So the pigeon orchid is interesting for many physiologists, developmental biologists, and molecular geneticists, as well as for plant breeders.

## **1.2 Literature review.**

### **1.2.1 Phenomenon and regulation of flowering**

Flowering is the most dramatic example of a programmed developmental change in higher plants. The transition from vegetative to reproductive development is a critical phase and represents a decisive stage in the life cycle (Metzger, 1987; Colasanti and Sundaresan, 2000). Flowering, or more precisely, reproductive development comprises of many independent processes which are highly coordinated. Control of this transition is very complex, frequently involving the integration of

environmental and developmental signals to ensure that flowering occurs at the best time of the year. This complex process includes several interrelated steps, each of which is influenced by various factors from both internal and external origins (Datta and Das, 2002). Two important factors which control flowering are temperature and light. These factors are perceived and transmitted to the nucleus to cause changes in gene expression that would lead to flower development (Sung *et al.*, 2003). In many plants, flowering cannot be induced despite being subjected to the proper induction conditions until a certain size or age is obtained. This is known as the juvenile phase. The length of the juvenile period can be as short as a few days or weeks in some herbaceous plants, or as long as forty years in some species of tree (Metzger, 1987). The transition from this juvenile to one permissive of flowering (adult or mature phase) is called phase change. In many species, the beginning of reproductive development is regulated by certain environmental factors i.e. temperature (vernalization) as well as day length (photoperiodism) (Goh *et al.*, 1982; Goh and Arditti, 1985).

#### **1.2.1.1 Temperature effects**

Vernalization is the phenomenon whereby low temperature treatment followed by placing in warmer condition promotes the initiation of reproductive development and it also refers to the induction or promotion of flowering by a low temperature treatment (Thomas, 1993). Thus, the perception and transduction of the environmental cue occurs during a cold period while floral development is displayed after placing the plants either under warmer temperature or growth-promoting temperature. Plant species which can be induced by this treatment,

a process sometimes called thermoinduction, will initiate flowers if they are maintained at the condition of low temperatures for a sufficient period of time. Optimum temperatures for vernalization are in the range 1-7 °C but sometimes slightly higher optima for plants from warmer regions. Numerous species also use seasonal variations as cues to coordinate the initiation of reproductive development. In addition to a general response to cold treatments, specific developmental stages may be particularly susceptible. Meristem differentiation, sporogenesis and gametogenesis are also vulnerable and stress at these stages leads to altered flowering times, sterility or abortion. Low temperature has been shown to induce flowering in some *Cymbidium* species and hybrids, *Phalaenopsis schillerana*.

#### **1.2.1.2 Photoperiodism and light intensity**

Photoperiodism was first reported in the early half of the 19<sup>th</sup> century. Briefly, the photoperiodic stimulus is perceived by phytochrome, pigment in leaves, which acts as a photoreceptor. This pigment can be inter-converted between two forms, Pfr and Pr. The Pr form, which absorbs red light at 660 nm, is converted to Pfr. This unstable Pfr form can be converted back to Pr in the presence of far-red light at 730 nm or can be slowly transformed in the darkness. It has been revealed that the Pfr is the more active form of pigment and it mediates most of the light response. The Pfr form can induce 'florigen' (flowering hormone) in the leaves. This florigen is translocated to the vegetative apical meristem and acts at this apex to convert the vegetative meristem into a floral meristem (Aukerman and Amasino, 1998; Datta and Das, 2002). It has been also suggested that a critical balance between Pfr and Pr is the genesis of the stimulus for the florigen production (Datta and Das, 2002). Day length

varies with the season in most parts of the world. The variations in day length are very small near the equator, and increase towards the poles. This means that plants such as orchids growing in different regions are subject to different day lengths or photoperiods which vary with the seasons. Until now, only a few studies in photoperiodic effect on orchid flowering have been reported and the number of species or hybrids examined, in particular *Cattleya*, *Cymbidium*, *Dendrobium*, *Paphiopedilum* and *Phalaenopsis*, is extremely small (Goh, 1992). As mentioned, most of the orchids which respond to short-day or long-day photoperiods are temperate in origin. Many tropical lowland species are either free flowering or flower gregariously at various times throughout the year. They are day neutral plants. The photoperiodic response of some orchids may be modified by temperature. For example, *Cattleya gaskellians* grown at 18 °C flowered under long-day but not under short-day conditions whereas at 30 °C they flowered under both long-day and short-day conditions (Goh, 1992).

The light intensity was also reported affecting the flowering. Although many tropical orchids are day neutral plants, they also exhibit peak flowering and seasons. For instance, *Vanda* Miss Joaquim was shown to be correlated with sunlight availability. *Aranda* hybrids require full sun for flowering and shading would delay or suppress the process (Goh, 1992). However, the effect of light intensity on flowering is less obvious in shade-loving orchids i.e. some species of *Dendrobium*, *Oncidium* and *Phalaenopsis*.

### 1.2.1.3 Hormonal control and flowering gradient

Based on observations under natural or commercial growing conditions, factors controlling flowering were reported in some orchids i.e. low temperature and plant growth regulators (PGR). However, the environmental conditions necessary for flower bud initiation might differ from those for bud growth and development. PGR could affect the flower development in both quantity and quality. Firstly, gibberellins (GAs) were shown to stimulate the mitotic activity in many plants within 24 hours of hormone application. A large number of species would respond mostly in long-day plants and cold-requiring plants. Gibberellic acid (GA<sub>3</sub>) brought about an increase in flower size and accelerated the flowering in some *Cymbidium* hybrids (Goh *et al.*, 1982). Secondly, cytokinins could also induce flowering in sympodial orchids, i.e. *Dendrobium* hybrids (Goh *et al.*, 1982; Goh, 1992). Benzyladenine (BA) caused an increase in length of inflorescence and numbers of flowers in *Dendrobium* cv. *Louisae* as well (Goh *et al.*, 1982). Not only cytokinins were demonstrated to initiate the floral bud in three *Dendrobium* hybrids but gibberellins were also used to induce flowering in *Bletilla striata*, *Cymbidium* and *Cattleya* hybrids. It was also reported that cytokinin effect was efficiently enhanced by the simultaneous application of GAs (Goh, 1992). The cytokinins (sometimes gibberellins) requirement might be directly involved in the control of vegetative and reproductive development in these orchids. So, cytokinins and gibberellins played an important role in the control of flowering. Thirdly, auxin was reported to inhibit flowering in monopodial orchids, i.e. other tropical hybrids, while cytokinin promoted flowering. The level of endogenous auxin controlled by apical dominance was also important in the floral development of *Aranda* cv. *Deborah* (Goh *et al.*, 1982; Goh,

1992). Its floral initiation could be stimulated by a decreased auxin level in stem tissues.

In addition, the important factor was the availability of nutrients required for the continued growth and development during flowering. Thus, a great deal of dry matter nutrients and water from the pseudobulb and leaves must be transported into the developing flower (Goh *et al.*, 1982). In some species, nutrition availability i.e. nitrogen, is closely bound to the flowering response but not for the majority of plants.

#### **1.2.1.4 Advanced understanding of flowering**

Understanding of flowering has changed during the last 30 years because of the availability of molecular biological techniques but many questions still need to be answered to understand the complex process of flowering. The classic concept of photoperiodic induction, hormone regulation and florigen used to form the basic knowledge of flowering. Consequent upon the advancement of technology, the present understanding suggests that the flowering events of higher plants are like the switching on of a cascade of genes, and the subsequent activation of genes in MADs box (Appendix A), resulting in the activation of the gene responsible for flowering (Levy and Dean, 1998; Datta and Das, 2001). At the molecular level, genes which 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, 1994). Therefore, rapid advances in the understanding of flower development can be expected.



## **1.2.2 Flowering process and reproductive development**

In general, the design of plant body is established during embryogenesis when the undifferentiated meristematic regions of root and shoot are set aside. The plant development, however, occurs postembryogenically through the reiterative production of 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). The change occurring at the SAM is controlled by environmental and endogenous signals. This SAM, however, is irreversibly committed to reproductive development once flowering commences. Besides, genes and processes involved in the transition to flowering are required for development of both reproductive initiation and maintenance. The timing of flowering is primarily influenced by either environmental factors in some species or the internal cues such as plant size or number of vegetative nodes in other species which are less sensitive to environmental variables. In the latter case, plants will not flower until they have reached the stage of ripeness to flower, termed ripe-to-flower (Metzger, 1987).

### **1.2.2.1 The vegetative phase converted to reproductive phase**

A series of genes are involved in the conversion of the SAM to inflorescence meristem (IM) and floral meristem (FM) (Datta and Das, 2002). According to the process of flowering, therefore, there are two processes which differ in some ways. In the first case, the whole process of flowering consists of two major steps; (1) the vegetative meristem is converted into reproductive meristem and (2) this reproductive meristem is differentiated into floral organs. To illustrate the transition,

the apical and lateral meristems are converted into the IM. This IM produces a series of reproductive lateral meristems called FM. Each FM ultimately gives rise to a single flower involving floral parts. In the second case, the flowering process is divided into two steps; (1) the first step is the initiation of flower primordium followed by (2) the development of this primordium into mature flower until anthesis. First, the entire indeterminate vegetative meristem (VM) is directly transformed into a determinate FM. Alternately, the VM is first transformed into an IM which then generates the FM. However, this IM neither produces floral organs directly nor generates leaves. It produces FM with bracts, or sometimes a mixture of FM and more IM, in the axils of the bracts. Second, the floral organs are produced in succession by the activity of this FM.

#### **1.2.2.2 Floral organization and floral organ identity**

The floral organization in each fold or whorl is determined by a unique combination of three organ identity genes or ABC-class genes (ABC model) (Datta and Das, 2002). Activity of Type A gene alone specifies sepals, whereas formation of petals requires both A and B gene activity. Stamens are formed by a combination of the activity of both B and C genes, whereas the activity of C alone specifies carpels (Appendix B). In a typical flower of angiosperm, four different floral organs, in particular, sepals (whorl 1), petals (whorl 2), stamens (whorl 3) and pistils (whorl 4) are initiated sequentially in the flanks of the FM to form the whorl of calyx, corolla, androecium and gynoecium, respectively. The sepals grow until they meet and encapsulate the other floral organs to form a closed bud. At the anthesis, the developmental program performed in the flower makes certain the more or less

synchronous condition of mature pollen grains, a receptive stigma, an egg and the central cells at the correct physiological stage for pollination and fertilization (Scott, 1994).

### **1.2.3 Experimental system and methods of developmental analysis**

#### **1.2.3.1 Plant materials and limitation**

Plants which can start to flower at the same time and are at the same stage of development during the growth period in non-inductive condition are required for many investigations, because sufficient uniform response can be obtained in order to be analyzed as a group when these plants are treated in inductive condition. However, unsatisfactory results may be achieved because of genetic heterogeneity of plant materials. To solve the problem, genetically uniform materials must be produced by clonal propagation or inbreeding before the work starts. In fact, the search for new remarkable plants for the experimental systems continues because the number of species suitable for flowering research is still quite small.

#### **1.2.3.2 Measurement methods of floral initiation and flowering**

Flowering measurements are always based on morphological changes at the apical meristem which are the results of several earlier processes occurring in different plant parts. Consider that (1) a plant is either flowering or not and (2) flowering is more abundant in a plant producing ten flowers than in the one producing only one flower. Thus, there is both a qualitative and a quantitative component (Bernier, 1981). To measure flower initiation, the method should evaluate

both the qualitative and quantitative characters of the developmental process. More than one method should be conducted with each species.

The simplest qualitative measurement of flower initiation is the proportion of plants that have initiated at least one flower after a given treatment. For the individual plants, the scoring is thus either + or -, and for the experimental population, the index of flower initiation is given by the percentage of + individuals. However, investigators must wait until macroscopic appearance of flower buds or even flower opening before determining this index. It is also suggested that the index of flower initiation should be determined by dissection microscope and microscopic examination at an appropriate time after experimental treatment. A disadvantage of this method is that the examined plants are destroyed.

The microscopic features of “floral stages” system and sometimes the macroscopic features of flower or inflorescence development, which are assigned increasing numerical values, are widely used. At some arbitrary time after the start of an experiment, the meristem of each plant within an experimental group is examined and, based upon its developmental stage, is assigned a number. The resulting values for the group are then averaged.

The methods used to quantitative flowering are numerous but they basically fall into five categories depending on the experimental design (Metzger, 1987). (1) The simplest is the percentage of plants that have flowered following particular treatments. (2) Measurement of the flowering response is to determine the number of buds, flowers, or flowering nodes on individual plants. (3) An experiment might choose to determine the number of leaves produced from the beginning of a treatment until flowering is observed. (4) The data could be obtained by determining

the time (usually in days or weeks) for flowering to occur. (5) At various times after the start of a treatment, the apex is examined microscopically and assigned a number, based on an arbitrary scale developed for that particular species. Thus, these numbered values assigned to different stages of apical development are scaled. This measure differs from the others in that it is destructive and therefore requires significantly more plants, but it does provide data on flower initiation.

So, a variety of methods have been used to measure flower initiation. Each method should evaluate both the qualitative and quantitative characters of the developmental process. Thus the most appropriate measuring techniques are chosen with respect to the species investigated, the aim and precision of the work, and the number of plants available.

#### **1.2.4 *In vitro* flowering: new tool for flowering studies**

Tissue culture technique has advanced rapidly in recent years. Somatic embryogenesis and organogenesis have become acceptable techniques for clonal propagation. Thus, the establishment of an efficient *in vitro* plant regeneration is the basis for various further studies, for instance, *in vitro* flowering system (Chang and Hsing, 1980) and genetic modification studies using genetic manipulation (Woodson, 1991). Besides, the *in vitro* flowering system, in particular based on somatic embryogenesis and organogenesis, is still useful to genetic analysis of expression of specific gene products during floral development. In addition, there are fewer variations in clonal propagation between plantlets obtained from tissue culture than seed-derived plantlets. In the latter case most of their characteristics are not uniform (Ishii *et al.*, 1998). Hence a method for high number explants with few unvarying

characters, suitable for subsequent experiment is desirable. Furthermore, the use of single genotype-derived cultures especially plants developed via embryogenesis, will not only solve the problems of genotype effects but also provide high regeneration, fast growing and uniform explants (Wang *et al.*, 2002).

*In vitro* flowering system provides a valuable system not only to study specific aspects of flowering or the whole mechanism of the reproductive process but also to shorten the breeding period of plant species. This system could be a new route and a valuable tool for flowering research (Yu and Goh, 2000). In the first case, it could be used for direct observation of a single cell change leading to different morphogenic patterns and to explore the factors involving flowering process as well as being extremely useful for studying the various control mechanisms in the flowering process (Goh, 1992). This approach has been applied with molecular techniques, in particular gene cloning and gene expression to study plant growth and development especially floral organ development (Yu and Goh, 2001). Although several homeotic genes controlling flower development have been isolated and characterized i.e. in *Antirrhinum majus* and *Arabidopsis thaliana* (Coen and Meyerowitz, 1991), the orchid floral homeotic genes involved in the floral initiation process still await isolation (Goh, 1992). In *Dendrobium* orchid, there has been only success in preparation of cDNA libraries from lateral organs at different developmental stages (Goh, 1992). It was revealed that the physiology of flowering of several orchids was different due to diverse origin. So, it is important that the classic knowledge of orchid flowering from various aspects is joined with modern approaches to analyze and control the flowering process (Yu and Goh, 2001). In the other case, the *in vitro* flowering system could offer potential value in breeding

programs by shortening the time for explants to initiate the first floral bud (Kerbaui, 1984; Duan and Yazawa, 1994; Wang and Zu, 1996). To illustrate, the early flowering of young seedlings in a flask have been occasionally reported but these seedlings took long periods to flower. They flowered usually after substantial vegetative growth. It was revealed that many orchid seedlings might grow for several years before flowering. The growth period from seed to flowering varies among species, hybrids or even individual plants derived from seed of the same capsule.

The *in vitro* flowering system was studied in some plants (Tefera and Chapman, 1992, for example, hybrid of *Petunia hybrida* and *Nicotiana plumbaginifolia* (Mulin and Tran Thanh Van, 1989), *Lycopersicon* spp (Liu and Li, 1989; Pierik *et al.*, 1994) and *Murraya paniculata* (Taha, 1997). There are some reports regarding the use of this system in some selected monocots, for instance, in bamboo (Nadgauda *et al.*, 1990; 1997; Chambers *et al.*, 1991), in many orchid species and their hybrids such as in *Oncidium varicosum* (Kerbaui, 1984), *Cymbidium ensifolium*, *Dendrobium candidum* (Wang *et al.*, 1981; Wang, 1988; Wang *et al.*, 1990 cited in Duan and Yazawa, 1994), *Doriella* tiny hybrids and *Phalaenopsis* (Duan and Yazawa, 1994; 1995). For instance, the seedlings of *Phalaenopsis* hybrids may flower within 2-3 years while *in vitro* flowering could be induced within 9 months (Duan and Yazawa, 1995). So, *in vitro* flowering could be applied to regulate or promote flowering in relatively short periods. It would be useful for both breeding programs and the orchid industry because of the decreasing cost of orchid production. Several orchids, however, have economic importance but few studies on their flower initiation and development have been undertaken until recently.

## 1.2.5 Characteristics of the pigeon orchid: *Dendrobium crumenatum* Sw.

### 1.2.5.1 Classification of *D. crumenatum* Sw.

The characters of the Orchidaceae separate them clearly from other families. The subdivision of the family has proved difficult and classification is diverse. The following classification, given in Willis, divides the family into three subfamilies and six tribes. Those of the Orchidoideae being separated principally on anther and pollinia characters. (Hickey and King, 1981; Mabberley, 1987):

Family Orchidaceae

(1) Subfamily Apostasioideae (triandrous orchids)

Tribe Apostasiaeae.

(2) Subfamily Cypridioideae (diandrous orchids)

Tribe Cypridioeae

(3) Subfamily Orchidoideae (monoandrous orchids\*)

Tribe Orchideae (orchidoid species)

Tribe Neottieae (neottoied species)

Tribe Epidendreae (epidendroid species)

Tribe Vandaeae (vandoid species)

\*More recently the monoandrous orchids have been subdivided into three subfamilies as following: (Dressler, 1986 cited in Endress, 1944)

Monoandrous orchids

Subfamily Neottiodeae

Subfamily Orchidoideae including Spiranthoideae

Subfamily Epidendroideae including Vandoideae



So, the *Dendrobium* is a monoandrous orchid and has been placed in subfamily Epidendroideae including Vandoideae (Endress, 1994) in the tribe Epidendreae (Kurzweil, 1987). This pigeon orchid has then been classified as follows:

### MONOCOTYLEDONAE (LILIOPSIDA)

Subclass	Liliidae
Order	Orchidales
Family	Orchidaceae
Subfamily	Orchidoideae (monoandrous orchid)
(Subfamily)	Epidendroideae including Vandoideae
Tribe	Epidendreae (epidendroid species)
Genus	<i>Dendrobium</i>
Species	<i>crumenatum</i>

#### 1.2.5.2 Gregarious flowering of *D. crumenatum* Sw.

The pigeon orchid, *Dendrobium crumenatum* Sw., is a tropical epiphytic plant belonging to the family Orchidaceae. It grows on every old tree in open country. Its life history is typical of many other Malayan orchids, and has many uncommon features, such as, its fragrant white flowers (Holtum, 1964; Went, 1990). This orchid produces inflorescences at intervals and flower buds are differentiated successively at the nodes of inflorescence axis. These flower buds remain dormant at early stage of development. They seem to start to develop again after a period of low temperature and bloom 9 days later. It flowers gregariously, *i.e.* all plants in a certain

area flower at the same time, throughout the year, but the blooms last for a full calendar day (Goh *et al.*, 1982). As all flowers open early in the morning and close in the late afternoon, this pigeon orchid is interesting for flowering research (Bernier *et al.*, 1981; Bernier *et al.*, 1993). This orchid species is perhaps the most interesting and widely studied example of flower induction (Goh *et al.*, 1982). Because of its gregarious flowering and its unique properties, this orchid has been an ideal plant for flowering research (Bernier *et al.*, 1981; 1993; Went, 1990). As a result of plants starting to flower at the same time and at the same stage of development, it is assumed that the time required to cover the final lap of development is constant in all individuals of the species. In specific terms, the four reasons to carry out research on this orchid are 1-: Its floral stages are synchronized. It flowers uniformly and sufficient for group analysis. 2-: the floral process is under relatively strict control of the environment, namely, temperature-dependent (Goh *et al.*, 1982; Goh and Arditti, 1985; Goh, 1992)) 3-: After the proper induction, all flowers go through the same conditions, and thus allow the investigator to focus the research on a limited number of flowers only 4-: flowers that open and close consistently at the same hour of the day, or that show a consistent rhythm of opening and closing, have preferentially been studied because development can be predicted to take place at a specific time, which facilitates such investigations (Endress, 1994). Like other flowering plants, an orchid must reach a certain stage of maturity before it can flower. Hence it is relevant that in *D. crumenatum* the flower buds remain dormant at a very early stage of development or they develop to a well-defined, and in every case the same, stage of advancement, at which point the growth stops. These buds thus grow to a certain size of the floral bud dormancy stage and then wait for a stimulus to induce full development.

### 1.2.5.3 Reproductive structures and development

#### 1.2.5.3.1 Structures and general characteristics (Figure 1)

Orchidaceae, based on the complexity of their floral architecture and fertilization mechanisms, is regarded as evolutionary advanced. There is not only considerable variation in floral form but also in when and how flowers are formed in their life cycle (Kurzweil, 1987a; 1987b; 1993; Morris *et al.*, 1996). *Dendrobium* orchid is monoandrous with monosymmetric flower. 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. One of the stigmas is modified into a structure called the rostellum. It is located above the functional stigmas and serves as a platform which bears the pollinarium under the anther cap. 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. The pollen grains were produced in masses known as pollinia. The fruit develops into a capsule. Another interesting feature of this monoandrous orchid is that the flower bud is borne with the labellum above the gynostamium. As the bud starts to open (or just prior to that), the pedicel twists 180 degrees in a process called resuspination. For *D. crumenatum*, the flower is produced from the chaff bract-clusters along the upper part of stalk-like plant body portions. The flower has a strong fragrance and is pure glittering white and the lip has a bright yellow disc (Figure 2).

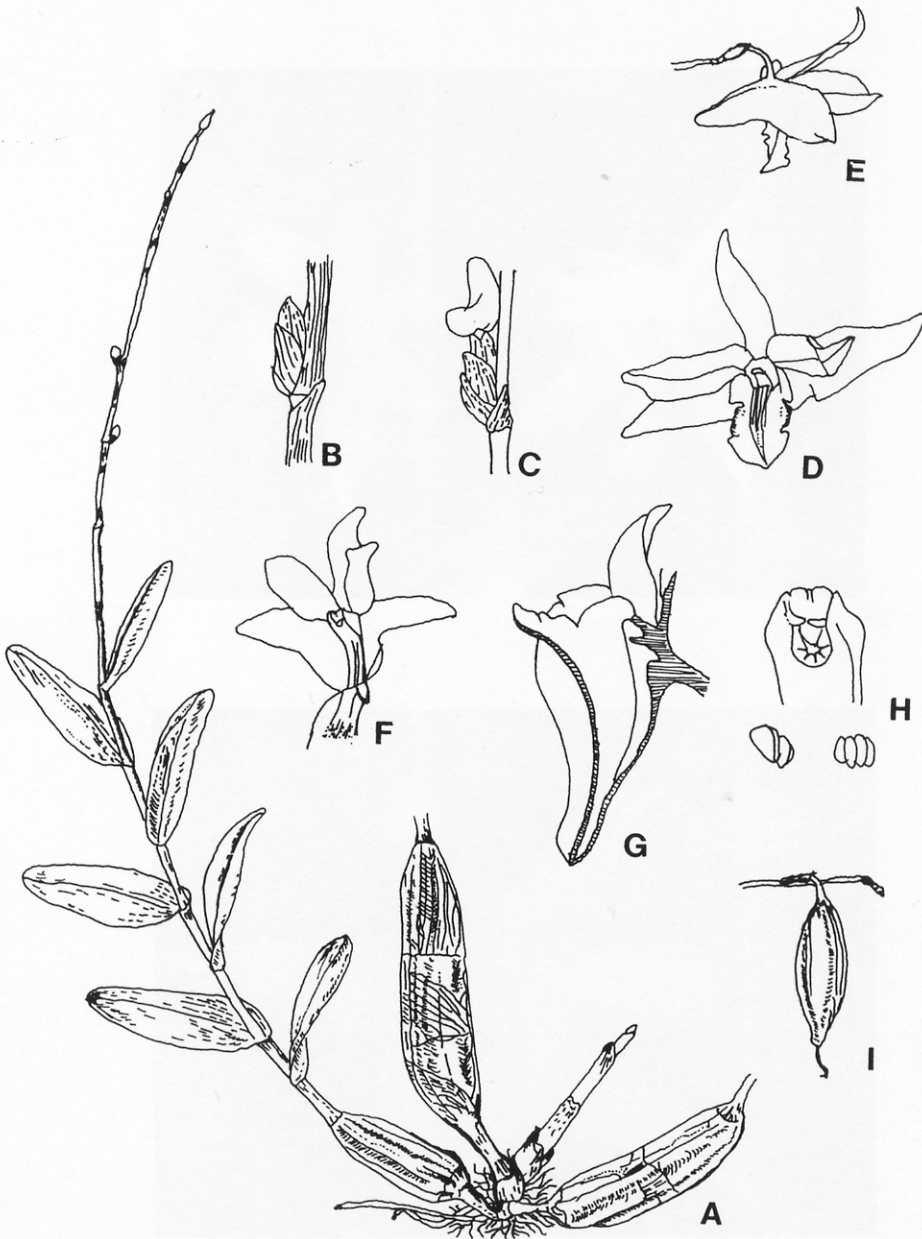


Figure 1 Morphological characteristics of *D. crumenatum* : Growing part of plant with a new shoot (A). Inflorescence breaking through leaf-sheath (B). Floral bud (C). Flower (D). Side-view of flower (E). Flower with lip pulled down, showing column-foot (F); vertical section of flower (G); Column and pollinia (h); (i) fruit (source: adapted from Holttum, 1964)



Figure 2 Flowers of *D. crumenatum* Sw. orchid show opened flowers (A) with pure glittering white and the lip has a bright yellow disc (B).

### **1.2.5.3.2 Initial differentiation of the flower primordium of monoandrous orchid**

Differentiation of floral primordium, and sequence of tepal initiation at early stage of flower development of Epidendroideae were investigated (Kurzweil, 1987). It was revealed that the flower primordium arises in the axillary position. Briefly, this primordium first appears as a transversely-stretched bulge and then grows up into an oval pad (Figure 3A). Prior to organ initiation a mound is formed, having a small transversal depression at its center (Figure 3B). In a great majority of orchids the labellum points upward in the bud (or non-resupinate position) and downward (or resupinate position) in the open flower (Goh *et al.*, 1982). This change in position results from a 180° twisting of the pedicel. First of all, in non-resupinate position, the first organ primordia becoming visible are those of the lateral sepals (S2, S3) on the adaxial side of the mound (Figure 3C). They are immediately followed by the lip (L) (Figure 3C). Until then the abaxial side of the mound has remained undivided. Its differentiation starts with the elevation of the lateral petal primordia (P1, P2) (Figure 3D). The last perianth member incepted is the medium sepal (S1). The anther primordium (A1) is differentiated from the abaxial half of the mound in an episepalous position (Figure 3E). While it grows up, the sepals (now being more or less equal in size) begin to close the flower bud (Figure 3F). After that, the anther grows in the upright position and flattens to a broad tongue-like structure. Observation on the late development such as gynostamium development, as well as the development of anther and pollinia were discussed in the first series devoted to the affinities of the Epidendroideae and Vandoideae described by Kurzweil (1987).

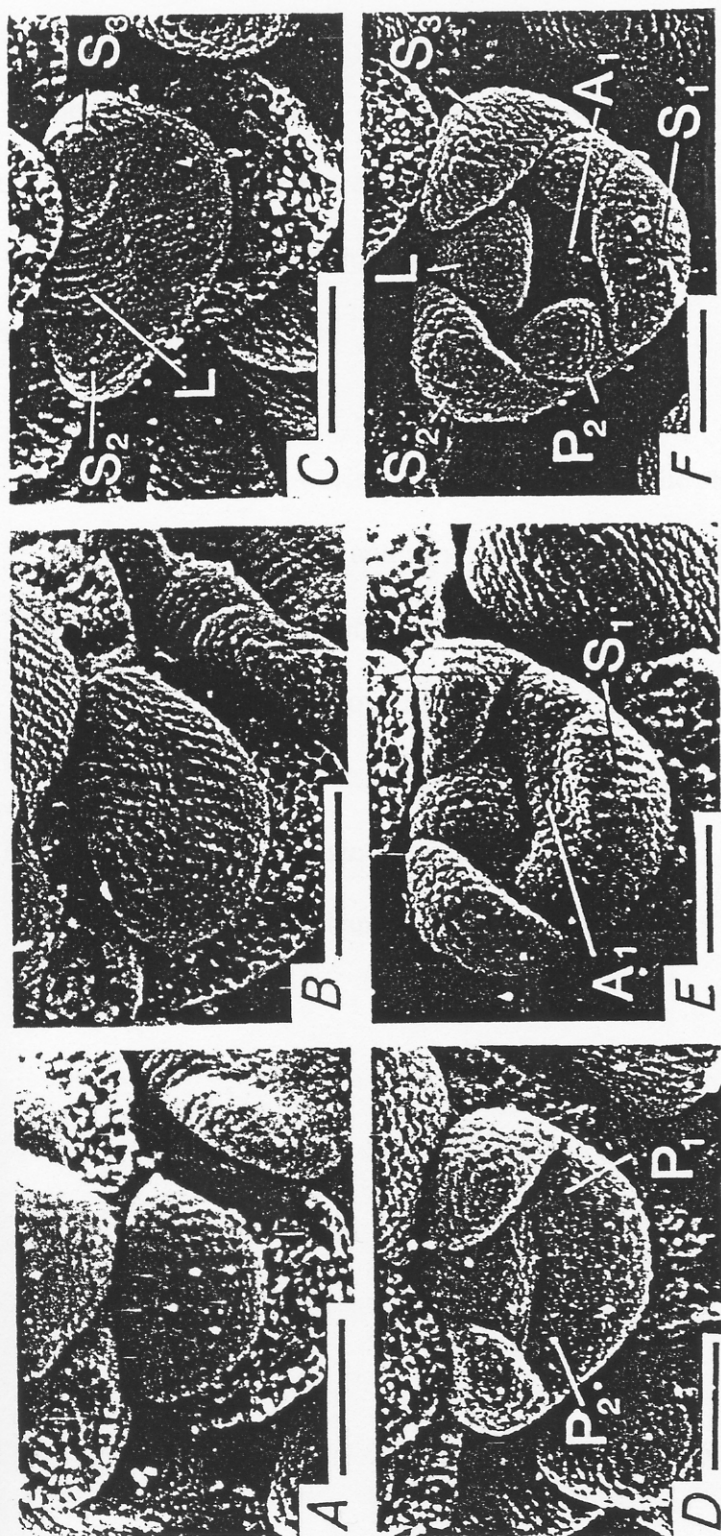


Figure 3 SEM micrographs showing the initial differentiation of the flower primordium of *Bletia purpurea* (Epidendroideae).

All primordia are shown in non-resuspinate position. Bar=0.1 mm.

(source: Kurzweil, 1987a)

### 1.3 Objectives of the research

The flowering pattern of the pigeon orchid, *Dendrobium crumenatum* Sw. needed to be investigated more extensively, so several experiments were conducted to answer the questions relating to flowering. The objectives of this research were described as the followings:

- 1 To investigate flowering behavior including the stage at which the floral bud responds to stimulus and time of pollen grain maturation.
- 2 To determine the natural stimulus and the effective physical and biological stimuli required for flowering.
- 3 To produce a high number of explants with a few unvarying characters required for subsequent experiment.
- 4 To induce floral bud and flowering *in vitro*.