5. CONCLUSIONS

5.1 Major findings of the thesis

The research was carried out to answer the questions regarding the flowering behavior of the pigeon orchid, *Dendrobium crumenatum* Sw. It introduced the natural and artificial stimuli which triggered the floral buds at the responsive stage to open. The main findings are briefly described as follows:

5.1.1 Flowering pattern, the responsive stage and pollen grain maturation.

The inflorescence of this orchid consisted of two to three floral buds at different developmental stages. Only the first (1^{st}) floral bud at the responsive stage could flower nine days after natural stimulus. Responsive stage, the stage at which the floral buds respond to the proper stimulus, was the most important factor determining the success in flowering. The 1^{st} floral bud showed the flower miniature completed with all floral parts was considered as the responsive stage. The two other floral buds developed sequentially after the same stimulus. The second (2^{nd}) floral bud developed the same as the 1^{st} floral bud and the third floral bud developed the same as the 2^{nd} floral bud. All buds stopped their development to wait for the next stimulus. The microsporocyte (2n) of the 1^{st} floral bud was still in a pre-meiotic stage. After natural stimulation, this cell developed rapidly to produce mature bi-nucleate pollen grains six days later or three days before anthesis. Both flowering process and pollen grain

development required the same stimulus to break the floral bud dormancy and to initiate the mature pollen grain.

5.1.2 A sudden drop in temperature required for natural flowering.

A sudden drop in temperature resulting from heavy rainfall triggered the flower blooming in nature. Only heavy rainfall in the early afternoon induced such a marked decrease in temperature. The difference in temperature drop after peak temperature within 2 hr was approximately 10 °C. The resting floral buds reached anthesis nine days (DTA=9) after heavy rainfall.

5.1.3 Low temperature required for the successful flowering.

Cooling was a physical factor triggering flowering. This orchid required exposure to a period of cold treatment to overcome the floral bud dormancy. Both cold treatment (CR) at 8° ± 2 °C in the dark and a preheating at 37 °C for 1 hr before cold treatment (HCR) stimulated the dormant floral bud to anthesis. Unfortunately, longer days to anthesis were obtained from both given treatments. The DTAs from CR and HCR experiment were 19 (DTA=19) and 21 (DTA=21) respectively, compared to nine (DTA=9) from natural stimulation, a big difference of 10-12 days.

5.1.4 BA and GA_3 applied separately were used as substitution for low-temperature requirement.

The biological factors, BA and GA_3, were active in flower triggering. BA and GA_3 applied separately induced the dormant floral buds to anthesis. The exogenous supply of BA at 10^{-1} - 10^{-2} M induced the resting floral buds to anthesis nine to 11 days later (DTA=9 and 11). Application of 10^{-2} M GA_3 caused dormant floral bud to anthesis 10 days later. These DTAs were nearly the same as DTA from natural stimulation. Unfortunately, the simultaneous application of BA and GA_3 was not successful to induce the flower opening.

5.1.5 The uniform explants developed through somatic embryogenesis and organogenesis.

Plant regeneration from callus culture could be efficiently established. In short, PGR and peptone were needed for callus proliferation phase, whereas the phase of PLBs formation required CW as an additive nutrient. PLBs themselves seemed to have the potential for further development. Addition of peptone to any media gave green, healthy and vigorous materials. It could be added into the media for all stages of plant development as a supplementary element. The uniform explants obtained from callus-derived PLBs developed through either somatic embryogenesis or organogenesis.

5.1.6 *In vitro* floral induction system and flowering under strict control of environment.

The length of the juvenile period could be as short as a few months using the *in vitro* floral induction system. The flower opening appeared after some induced plants were transplanted to the natural greenhouse conditions. The induced plant could flower within one year compared to six to seven years for a natural plant, an enormous difference of five to six years. However, culture for a longer period of time in a liquid medium caused the malformed floral buds.

5.2 Potential uses

This research is directed at understanding flowering in the pigeon orchid, *D. crumenatum* Sw. Its flowering pattern was examined from several aspects in a solid package to answer the questions which are needed for advanced research in the future. This orchid could be characterized as a model of the cold-requiring plant for many aspects of research in flowering, particularly for floral initiation, floral induction, floral development and, programmed cell death of the plant. Due to genetically control of floral development the flowering process can be studied for the responsible genes. Then the pattern of gene expression can be mapped and the consequence of gene loss or gain of functions can be assessed. Finally, access to molecular functions of floral developmental genes will be available. The desirable genes could be isolated to manipulate new plants suitable for orchid production. In addition, the knowledge obtained is very useful and critical for future flowering research in orchid.