CHAPTER 5

CONCLUSION AND DISCUSSION

Studies on character and floral development of *Hippeastrum* carried out in this research derived from the problems disclosed by researchers working on *Hippeastrum* improvement and commercial *Hippeastrum* growers. Since *Hippeastrum* is rather diversified in species and abundant commercial varieties had evolved through such diversity, the future as popular ornamentals of this plant is thus undoubtful. To maintain its status in commercial circle, it is necessary that new varieties of trendy attractions are regularly released. As for this purpose, advantages in developing new varieties exist since a large number of varieties and species are accessible. But, capacities of interhybridizing, specific or generic, are yet to be overcome.

The research carrying out here covered some physiological aspects to support such attempts. A part of this research concerned biological studies of pollen morphology, anatomy of some plant parts and chromosome investigation, aiming at characterization of the plants to obtain possible identities of the parent varieties as well as those of the hybrids. These examinations were done with both diploid and tetraploid varieties to develop protocol procedures in use for further research. Another part of the studies involved floral development of the plant providing better understanding for the growers in dealing with the bulbs beneficially and for the breeders in handling the plants to their maximum capacity of hybridization. Discussion and conclusion of the experiments are conducted here in accordance with objectives stated above, as follows:

1. Pollen morphology

It was found from morphological investigation that the pollens of 7 varieties of *Hippeastrum* obtained mutual features of ellipsoid monad, heteropolar, monosulcate and of bilateral symmetry. The size of pollen grains were large to very large, with the length of 88.20-121.24 μm on the polar axis and 30.25-38.36 μm on the equatorial

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with the size of 88.20-112.15 µm in length of polar axis and 30.25-37.13 µm in length of equatorial axis. The exine was of reticulated type with different sculpturing, depending upon varieties.

Although the results concluded here coincide with those reported by Anderson *et al.* (2007) that the features of *Hippeastrum* pollen were similar to those of Amaryllidaceae, but it can be clearly seen in this research that under SEM the exine sculpturing can differentiate individual variety from others. Therefore, specific exine feature can literally clasify *Hippeastrum* to variety level and is able to be used as varietal identity.

As for the size of the pollen, it can be considered here as theretically stated (Garber, 1972) that pollens of tetraploid varieties are larger than those of the diploids from the sizes of SP-Pink /SP-Red vs Night Star and Tango.

2. Anatomical characterization

2.1 It can be concluded from the research towards anatomy of leaf, petal, peduncle and pedicel of 4 varieties of *Hippeastrum* that the internal structure of these organs reflects those of other plant species belonging to Amaryllidaceae as reported by Khobkhet (1971). Transverse sections of the leaves exposing the mesophyll of parenchymatous tissue undifferentiated into palisade and spongy layer as found here agree with the results stated by Vitayasak (1996) in her studies of *Hippeastrum* and in the texts written by Bootrat (1989) and Esau (1977).

2.2 Practical tips were achieved through staining tests that single stain of haematoxylin was more precise than double stain of safranin and fast green since the tissues used in this study were of primary structure.

2.3 Special interests were made towards stomata appeared in the surfaces of different organs since stomata could reflect several aspects both biologically and physiologically. Firstly, it can express identification of the plants to a certain extent by its shape and appearance of subsidiary cells (Esau, 1965). Secondly, the size of stomata of the plant belonging to the same species can determine their ploidy levels. Thirdly, the size of stomatal pores in complement with stomatal frequency in the surfaces of particular organs are supposed to relate to transpiration capacity of individual organs in certain situations.

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Results of stomatal examination in this research could serve the query in all aspects stated above. It was seen that all 4 varieties constructed the same structure of stomata, being anomocytic with no subsidiary cells. Distribution of stomata was also similar in those varieties, i.e. scattering all over the surfaces of the plant body and found in both upper and lower epidermis of leaves and petals. Frequency of stomatal appearance was highest, among the 4 organs, in leaves then reduced successively in pedicel, petal and peduncle. To this extent, it could be seen that *Hippeastrum* contained the same structure of stomata as those of monocotyledon plants as stated by (Soontornchainaksaeng, 2006; Meidner and Mansfield, 1968; Fahn, 1990). The fact that stomata of *Hippeastrum* were formed in most part of the plant body with differences in appearing frequency could lead to the question towards relating index involving transpiration capacity vs density of stomata appearing on particular organs affecting post harvest or keeping quality of the plant/flower.

As for justification of stomatal size over the ploidy levels of the plant of the same species (Krishnaswami and Andal, 1978; Przywara *et al*., 1988; Mishra, 1997; Aryavand *et al*., 2003), it could be gathered from this study that diploid varieties of SP-Pink/SP-Red obtained stomata of smaller size than those of the tetraploid Apple Blossom/Susan.

3. Chromosome investigation

Although chromosome investigation of *Hippeastrum* is nothing new here since it has been carried out by several scientists (Arayakijcharoenchai (2000), Chawalid (2003), Choticasatian (2002), Patanakanog (1999), Sirikhum *et. al.* (2008), Sopinvetaya (1980), Sudsa-nguan (2001) and Vitayasak (1996), but specific technique of preparing the root tip for chromosome study obtained from this experiment can be considered as the prototype procedure. It serves will with certain accuracy making the practice consumed least time and less expenses. It also yields the chromosomes at their maximum contraction convenience efficient karyotypic studies. With the aid of carbol fuchsin solution prepared through modified recipe successfully conducted here staining of the chromosome is much improved.

Another tip concerning root tip preparation is that staining ability of the tissue depends much on the temperature and timing during the step of maceration. Constant

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temperature and adequate duration of maceration provide better staining. On the contrary, over maceration can prohibit stainability of the tissue.

Suitable procedure is concluded as 1) taking the sample during 9.30-10.00 a.m., 2) pretreating the root tip in PDB for 48 hours, 3) macerating in 1N HCl for 5 minutes and 4) staining in carbol fuchsin for 24 hours.

Chromosome counts of diploid varieties were 2n = 22 while tetraploid varieties were 2n = 44. Their results are similar to the reported by Arayakijcharoenchai (2000), Chawalid (2003), Choticasatian (2002), Patanakanog (1999), Sudsa-nguan (2001) and Vitayasak (1996).

4. Floral development

Flowering behavior of bulbous is unique. Development of the flower since the stage of initiation through to completion of floral part formation occurs inside the bulb (Okubo, 1993). Formation of the flowers of *Hippeastrum* is no exception, according to the studies carried out by several authors (Arayakijcharoenchai, 2000, Sudsa-nguan, 2001). Investigation of floral development organised in the research, although following the same paths of examination, but particular interests in certain stages of such develop were emphasized, aiming application of the knowledge towards the fields of hybridization and of bulb handling for commercial production.

4.1. Formation of floral bud

As in typical *Hippeastrum* bulbs, varieties tested in this research followed the same pattern of flower formation. Inside the bulb, at every forth scale axil away from the apical vegetative bud its axillary bud was able to become reproductive and develop into an umbel spike. But, significance occurring here in this study was that diploid bulbs of various sizes bore more growing floral buds than those of the tetraploids and that large bulbs (20/25) of tetraploid varieties accommodate only 1 floral bud or 2, at the most. This showed that minimum flowering size bulb of the diploid varieties were much smaller (10/12), reflecting somewhat higher capacity to form flowers. Moreover, diploid bulbs, as discovered here, contained more bulb scales than those of tetraploids of the same bulb size, indicate higher number of scale axils capable of initiating the floral bud in proper or controlled environments. These

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physiological facts regarding specific potential of flower formation literally inspired further study of introducing this behavior into improved commercial varieties.

On the other hand, basic information concerning development of the floral buds inside diploid bulbs is useful for the bulb growers as well as the bulb dealers to handle the bulbs cautiously to avoid possible desiccation damages in interrupting the growth and development of the small floral inside the bulbs. This type of caution is important since new diploid and triploid hybrids are coming into attractions especially in sophisticated florists in towns as it is today in foreign markets. Likewise, for hybridization purposes, the same cautions should be taken with the parent bulbs of small-flowered species for the sake of the bulb handing.

5. Pollen viability and storage

Viability tests of the pollens of 7 varieties were aimed at searching of the most suitable time of the day for fruitful pollination resulting high percentages of successful fertilization of crosses.

Methods of efficient viability test were investigated and it occurred that staining the pollens in acetocarmine dye or germinating the pollen in culture media both yielded acceptable results and were marked as efficient methods of viability tests of the pollens as also reported Chawalid (2003) and Patanakanog (1999).

The pollens of experimental varieties germinated well in examination trials during the period of 6.00-10.00 a.m. of which also reported by Chawalid (2003). Thus, suggestions are made based on this result that pollination of parent plant at should be pollinated during such time when the triploid is not so high while the RH is adequate.

As for pollen storage, the results gained from this study is very useful since it revealed that the pollens of 7 varieties can be stored for rather long period of time at $5^{o}C$ with satisfactory germination percentages.

6. Embryo development

Examination of embryos inside hybrid seeds at different stages of development were planned to be carried out since the early days of pod development. But such investigation was not achieved due to technical problems in preparing ovary

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tissue for sectioning. The objects were too large to be totally infiltrated, thus impossible to obtain perfect sections.

The only manageable examination was that of the embryos extracted from the hybrid seeds observed under light microscope. These embryos resembled those of monocotyledonous plants.

Failure in this experiment suggested that other means of obtaining better sections of young embryos inside developing hybrid seeds are needed since progressive development of those embryos can reveal information concerning physiological aspects of growth and development of the seeds obtained from different crosses useful crossability for analysis of interhybridization among diversified varieties.

7. Application

Gathering from research results, application could be compiled in supports of hybridization activities and *Hippeastrum* production management, in accordance with the research objectives in 7 categories as stated below:

7.1 Varietal identity through characterization

7.1.1 Extracting from characterization results, the most prominent identification of the variety for parent plants or hybrids alike is that of exine sculpture of the pollen.

7.1.2 Chromosome number and chromosome configuration could be useful for the breeders and variety releasers, in term of chromosomal identity. As in polyploid breeding, quite a number of large-flowered hybrids are triploid and anueploid derived from of tetraploid, for instance, the variety Red Lion, being an anueploid of 2n = 4x-1 = 43 (Vitayasak, 1996). Varieties such like this, being different in special manner, as anueploid, having less number of chromosome from the regular set. The monosome of *Hippeastrum* can easily be identified from its karyogram since metaphase chromosomes can simply be obtained from root tip squash technique developed from this study.

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7.2 Estimation of parent varieties through anatomical structure

Anatomical structure such as epidermal texture, stomatal frequency, tissue systems of ground and vascular of certain organs can be used as estimating indices in terms of tolerance to environments, strength of internal supportive elements, conducting capacities of vascular tissues, relating to physiological phenomenon affecting growth and post-harvest quality of the parent plants. These aspects can be evaluated from transverse sections and epidermal peels prepared by the methods and special techniques simplified in this research, as proposed in the manuscript.

Remarks are made on the tissue structure of supportive organs the peduncles, of which are the part of the stem erecting the flowers. Parent varieties obtaining strong pedicels with less stomatal frequency should posses superior characters. The peduncle is also another important supportive organ, especially when it is hollow inside, thus have less area of parenchymatous tissue to provide turgidity.

Notes are also granted on the ground tissue system of the petals since it was observed from this study that the petals of *Hippeastrum* contain large air spaces giving small areas of water holding mesophyll to provide adequate turgidity to the petal tissue, even in the large-flowered tetraploid hybrids, let alone the thin cuticle layers above the epidermis.

Estimation of post-harvest properties belonging to various parent species can be carried out through the simple techniques of antomical studies. Estimating and justifying of the varieties prior to selecting of parent varieties could save the time, expenses and labour in conducting the hybridization while expectation of the hybrid characters could also be made.

7.3 Orientation and fertility of floral buds

It is notable that commercial tetraploid bulbs contain only 1 or 2 floral buds reflecting flowering capacity of the varieties. But, in diploid small-flowered bulbs a certain number of the floral bud appeared inside the bulb indicating better flowering capacity, reflecting more number of flower per plant available for pollination and fertilization.

As for growers who commercially produce small-flowered species/varieties, being aware of the nature of varieties, benefits can be made from the following

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information. All of the floral buds oriented in those bulbs are fertile at the time of growing, as gathered from the study. Proper handling of the bulbs, avoiding drying out during dormant period should preserve those floral buds. With extra nutrient feed to these bulbs stimulation of small-sized floral buds could occur, giving more blooming inflorescences emerging from the bulb.

7.4 Germination tests and storage of pollens

As mentioned earlier, germinability of the pollens can be checked by simple methods with reliable accuracy. Pollination at proper time could thus be estimated from these tests. Pollen storage allows the breeders to work on hybridization of the parents having different blooming periods.

7.5 Embryo rescue of polyploid seeds

It is unfortunate that the experiment concerning embryo development of hybrid seeds was unsuccessful, otherwise useful information would be granted regarding to the matter. Nevertheless, practical improvement should be commenced to solve the technical problems.

Application approaches suggested above should literally indicate achievement in conducting the research on characters and floral development of diploid and tetraploid *Hippeastrum*. Nevertheless, further studies should be made from the basic information produced from this research to strengthen commercial production of *Hippeastrum*, benefitting the growers in the lowlands.

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