

## CHAPTER 6

### CONCLUSIONS

1. Genetic similarity of 36 samples from 30 species of the genus *Phalaenopsis* and related genera, *Doritis* and *Kingidium*, was evaluated based on RAPD technique using 20 decamer primers. Six primers, OPAK10, OPD03, OPF01, OPF02, OPF09 and OPF14, showed polymorphic DNA bands with high resolution and produced 82 polymorphic bands ranging in size from 223 to 2,300 bp. The genetic distance using 6 primer combinations ranged from 0.14 - 0.79. The results from UPGMA cluster analysis using 6 primer combinations among 36 samples could distinguish and divide the genus *Phalaenopsis* and related genera into 9 major groups at genetic distance of 0.2, which were almost correspondent to the analysis of genus *Phalaenopsis* by morphological characteristics by Christenson (2001). The bootstrap confidence values for clusters were in the ranges of 35.7 - 92.5 %. Genetic distance values among 8 sections of *Phalaenopsis* and 2 related genera, *Doritis* and *Kingidium*, ranged from 0.07, between section *Zebrinae* and section *Amboinenses*, to 0.46, between section *Zebrinae* and section *Proboscidioides*. The dendrogram from UPGMA cluster analysis of 6 primer combinations could distinguish and divide the genus *Phalaenopsis* and related genera into 2 major groups at genetic distance of 0.15. The results showed that 2 related genera, *Doritis* and *Kingidium*, should be treated as genus *Phalaenopsis*.

2. Crossabilities among twenty-four interspecific crosses, i.e. 21 intersectional and 3 intrasectional, and 20 intergeneric crosses were tested. It was found that

only 7 crosses could yield hybrids, which showed low crossability, 2.65 %, among the intersectional, intrasectional and intergeneric hybridizations. Genetic distance values ranged from 0.15 to 0.67 among 44 crosses, whereas the 24 crosses that yielded fruit set had genetic distance values ranged from 0.15 to 0.58. The results showed genetic similarity between parents of 7 crosses as follows: 4 intersectional hybridizations, 1) section *Phalaenopsis*: *P. amabilis* x section *Polychilos*: *P. cornu-cervi* had the genetic distance value of 0.35, 2) section *Phalaenopsis*: *P. schilleriana* x section *Polychilos*: *P. cornu-cervi* had the genetic distance value of 0.27, 3) section *Zebrinae*: *P. violacea* x section *Polychilos*: *P. cornu-cervi* had the genetic distance value of 0.48 and 4) section *Zebrinae*: *P. violacea* x section *Amboinenses*: *P. javanica* had the genetic distance value of 0.35, 1 intrasectional hybridization, section *Parishianae*: *P. gibbosa* 1 x *P. parishii* had the genetic distance value of 0.15 and 2 intergeneric hybridizations, genus *Doritis*: *D. pulcherrima* ‘dwarf’ x genus *Phalaenopsis*: *P. equestris* had the genetic distance value of 0.44 and 2) genus *Doritis*: *D. pulcherrima* ‘dwarf’ x genus *Kingidium*: *K. minus* had the genetic distance value of 0.41. After culturing under aseptic condition for 6 months, the greatest number of hybrid seedling was 1,000 plantlets obtained from *P. schilleriana* x *P. cornu-cervi*, whereas the least number, 40 plantlets, was found in cross *P. violacea* x *P. javanica*. After transplanting for six months, the highest survival rate of *P. schilleriana* x *P. cornu-cervi* was 81.25 %, whereas the lowest 43.33 % survival plant was found in cross *P. gibbosa* 1 x *P. parishii*.

3. The segregations of hybrid phenotypes of 3 compatible crosses were evaluated. The results showed that most progenies from cross *P. schilleriana* x *P. cornu-cervi* had dark green leaves which were similar to female parent, whereas

they had star-shaped flowers which were similar to male parent and the flower colors showed transgressive segregation of the two parents. Most progenies from crosses *D. pulcherrima* 'dwarf' x *P. equestris* and *D. pulcherrima* 'dwarf' x *K. minus* had dark green leaves and dark pink flowers with four pollinia which were similar to female parent while the flower size and shape were similar to male parent. Based on RAPD technique, the 20 decamer primers were used to test the genetic similarity of parents and their 10 progenies of 3 compatible crosses. The number of primers giving polymorphic DNA bands varied among crosses. Suitable primers for each cross could be described as follows: 6 primers, OPAK10, OPD03, OPF01, OPF02, OPF09 and OPF14, for cross *P. schilleriana* x *P. cornu-cervi*; 4 primers, OPAK10, OPF01, OPF02 and OPF09, for cross *D. pulcherrima* 'dwarf' x *P. equestris* and 5 primers, OPAK10, OPD03, OPF02, OPF09 and OPF14 for cross *D. pulcherrima* 'dwarf' x *K. minus*. The DNA fingerprints were presented showing polymorphic RAPD markers from either parent that appeared in hybrid banding pattern.

Parental lines, *P. schilleriana* and *P. cornu-cervi* and their 10 progenies were analyzed with 6 primers, OPAK10, OPD03, OPF01, OPF02, OPF09 and OPF14. Banding patterns showed polymorphic DNA bands in the ranges of 422 - 2,050, 275 - 1,602, 394 - 1,500, 358 - 2,364, 310 - 1,411 and 373 - 1,582 bp, respectively.

Parental lines, *D. pulcherrima* (miniature plant) and *K. minus* and their 10 progenies were analyzed with 5 primers, OPAK10, OPD03, OPF02, OPF09 and OPF 14. Banding patterns showed polymorphic DNA bands in the ranges of 210 - 1,200, 310 - 1,362, 118 - 1,000, 382 - 1,250 and 623 - 1,579 bp, respectively.

Parental lines, *D. pulcherrima* (miniature plant) and *P. equestris* and their 10 progenies were analyzed with 4 primers, OPAK10, OPF01, OPF02 and OPF09.

Banding patterns showed polymorphic DNA bands in the ranges of 262 - 425, 100 - 574, 127 - 405 and 150 - 519 bp, respectively.

Genetic similarity between parental lines and their 10 progenies using principle component analysis (PCA) was analyzed according to RAPD banding patterns with the NTSYS-pc version 2.01 program. The results showed that all 10 progenies of cross *P. schilleriana* x *P. cornu-cervi* were widely distributed between female and male parents. Eight progenies of cross *D. pulcherrima* 'dwarf' x *P. equestris* were clustered along with male parent whereas the other 2 plants were distributed between female and male parents. In another intergeneric hybridization, 2 progenies of cross *D. pulcherrima* 'dwarf' x *K. minus* were clustered along with female parent whereas 1 plant was clustered with male parent. The other 7 progenies were widely distributed between female and male parents.

4. AFLP technique was used to trace specific marker that linked to the flower color pattern of *P. cornu-cervi*. Among 64 primer combinations of 8 *EcoRI* primers and 8 *MseI* primers used in the study, *EcoRI*+AG/*MseI*+CAG primer combination yielded two specific DNA bands, which were found only in reddish brown flower and yellow flower with reddish brown bar and spot groups. DNA sequencing of two DNA fragments showed 229 and 278 bases. DNA sequences were analyzed and compared with DNA sequences database in NCBI GenBank. After BLAST searches of the sequences, 229- and 278-base sequences showed 78.7 % DNA sequences homology to the *Citrus reticulata* AFLP marker AFLP-4 genomic sequence which linked to the seedless trait. The results of this study concluded that AFLP marker could assist separating of reddish brown flower and yellow flower with reddish brown bar and

spot groups from pure yellow flower (no spot or bars) group. However, specific marker related to flower color pattern was not found.

In conclusion, the relationship of crossability, morphology and molecular work might be a useful tool for future classification. RAPD marker could provide information on classification of genus *Phalaenopsis* and its related genera, *Doritis* and *Kingidium*. The polymorphic DNA bands could be used to identify the genetic similarity between parental plants and their hybrids of interspecific and intergeneric hybridization. AFLP marker could assist to separate reddish brown flower and yellow flower with reddish brown bar and spot groups from pure yellow flower (no spot or bar) group. Further study, using other molecular technique such as cDNA-AFLP might be able to confirm the result of this study.