CHAPTER 5

DISCUSSIONS

5.1 Analysis of genetic relationship of genus *Phalaenopsis* and related genera, *Doritis* and *Kingidium*, by RAPD technique

The suitable primers for RAPD technique were OPAK10, OPD03, OPF01, OPF02, OPF09 and OPF14. These six primers showed 82 polymorphic DNA bands with high resolution. The amplified fragments ranged in size from 223 to 2,300 bp. Genetic distance among the 36 samples from 30 species of *Phalaenopsis* and related genera, *Doritis* and *Kingidium*, was evaluated using POPGENE version 1.32 program (Yeh *et al*., 1999). The results showed genetic distance values of genus *Phalaenopsis* ranging in size from 0.14, between *P. violacea* and *P. violacea* var. *sumatra*, and *P. violacea* and *P. bellina*, to 0.79, between *P. corningiana* and *P. lowii*. Comparison among 25 *Phalaenopsis* species, and two related genera, *Doritis* and *Kingidium*, displayed the genetic distance values in the ranges of 0.29, between *D. pulcherrima* 'dwarf' and *P. javanica*, to 0.67, between *K. philippinensis* and *P. amboinensis* 1. 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*. Based on the UPGMA cluster analysis, the dendrogram of 6 primer combinations could distinguish and divide the genus *Phalaenopsis* and related genera into 9 major groups which were almost correspondent to the analysis of genus *Phalaenopsis* and their relatives by morphological characteristics by Christenson (2001). The bootstrap

confidence values for clusters were in the range of 35.7 - 92.5 %, the low confidence values might be due to an artifact of the clustering technique rather than a reflection of the genetic relationship among species.

Group 1: The members of this group came from 2 sections which could be divided into 3 subgroups. Subgroup 1 consisted of *P. javanica* and *P. micholitzii* from section *Amboinenses*, and *P. sumatrana* from section *Zebrinae* (Sweet, 1980). Based on molecular data and flower color, *P. javanica* and *P. sumatrana* were more alike, the flowers of these two species have spots on petals and sepals, and they are also found in the same location (endemic to Indonesia) whereas flower of *P. micholitzii* is creamy white without any spot and its origin is from Philippines (Christenson, 2001). Subgroup 2 consisted of *P. hieroglyphica, P. pulchra* and *P. mariae*, which belong to section *Zebrinae*, the members of this group have star-shaped flower and they are endemic to Philippines (Christenson, 2001). Subgroup 3 consisted of *P. violacea, P. violacea* var. *sumatra* and *P. bellina* from section *Zebrinae*. The flowers of this group have star-shaped and fragrance, and their origins are Malaysia and Indonesia (Christenson, 2001).

Group 2: The members of this group came from 2 sections which could be divided into 2 subgroups. Subgroup 1 consisted of *P. viridis* and *P. fuscata* from section *Fuscatae*. The flowers of these two species have the petals equal to the sepals and they are found in Philippines (Christenson, 2001). Subgroup 2 consisted of *P. amboinensis* 1, *P. amboinensis* 2 and *P. venosa* from section *Amboinenses*, and *P. corningiana* from section *Zebrinae* (Sweet, 1980). All species in this group have star-shaped with spotted flowers. The molecular data supported that these species should be put in the same section. Data from chromosome size study also indicated the

similarity of these species (Kao *et al*., 2001). However, these three species were placed in different sections due to their geographic distribution, *P. amboinensis* and *P. venosa* are found in Indonesia whereas *P. corningiana* is found in Borneo (Christenson, 2001),

Group 3: This group had 2 species, i.e. *P. equestris*, *P. lindenii* 1 and *P. lindenii* 2, from section *Stauroglottis* (Sweet, 1980). The flowers of these three species have the petals equal to the sepals and they are found in Philippines (Christenson, 2001).

Group 4: There were 5 species which belonged to 2 sections: *P. amabilis*, *P. aphrodite, P. schilleriana* and *P. philippinensis* from section *Phalaenopsis*, and *P. cornu-cervi* from section *Polychilos*. Even though *P. cornu-cervi* has star-shaped and yellow flower which differs from other members of section *Phalaenopsis* that have large and round flower with the petals larger than the sepals (Sweet, 1980). The results from molecular comparison revealed close relationship of *P. cornu-cervi* to the section *Phalaenopsis*. The members of this group were similar to the report of Tsai *et al*. (2003) based on the internal transcribed spacer 1 and 2 (ITS 1 and ITS 2) of rDNA. In this study, compatibilities of crosses *P. amabilis* x *P. cornu-cervi*, and *P. schilleriana* x *P. cornu-cervi* also suggested that *P. cornu-cervi* had close relationship to the section *Phalaenopsis*. In addition, the chromosome size study of Kao *et al*. (2001) also indicated that the species in section *Phalaenopsis* and *P. cornu-cervi* had small chromosome sizes.

Group 5: There were 2 species which belonged to the genus *Kingidium*, *K. delisiosa* and *K. philippinensis*. The members of this group have four pollinia. The

species of this group are found in widespread from Sri Lanka, India, Thailand, Sulawesi to Philippines (Christenson, 2001).

Group 6: All species belonged to the genus *Kingidium*, *K. minus* and *K. braceana*. The members of this group have four pollinia. Although this group is different in geographic distribution in that *K. minus* is endemic to Thailand whereas *K. braceana* is found in Bhutan and China (Christenson, 2001), result from molecular data showed that 2 species had close relationship. In addition, the classification of Christenson (2001) indicated that *K. minus* and *K. braceana* should be put in the same subgenus *Aphyllae*.

Group 7: The members of this group consisted of *Doritis pulcherrima*, *D. pulcherrima* 'dwarf' (miniature plant) and *D. pulcherrima* var. *buyssoniana* (tetraploid plant). The members of this group have four pollinia. The first two samples are found in widespread from Northeast India and Southern China throughout Indochina to Malaysia, Indonesia and Thailand while *D. pulcherrima* var. *buyssoniana* is found in Indonesia and Thailand (Christenson, 2001).

Group 8: There were 2 species which belonged to 2 sections: *P. lowii* from section *Proboscidioides* and *P. mannii* from section *Polychilos*. The members of this group are different in growth habit, morphological characteristics and geographical distribution. *P. lowii* is deciduous and has four pollinia. The petals are broader than the sepals and it is found in Myanmar and adjacent western Thailand. On the contrary, *P. mannii* is evergreen and has two pollinia. The petals are equal to the sepals and it is found in Northeast India, Nepal and China to Vietnam (Christenson, 2001).

Group 9: This group was from section *Parishianae*, i.e. *P. gibbosa* 1, *P. gibbosa* 2, *P. lobbii* and *P. parishii*. The members of this group have four pollinia.

They are deciduous and found in Southeast Asia (India, Vietnam, Myanmar and Thailand) (Sweet, 1980; Christenson, 2001).

Genetic distance 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 (Figure 25).

Group 1: Consisted of 7 sections from genus *Phalaenopsis*, i.e. section *Amboinenses*, *Zebrinae*, *Phalaenopsis*, *Parishianae*, *Polychilos*, *Fuscatae* and *Stauroglottis*, and genus *Kingidium*. The members of this group have two pollinia except for genus *Kingidium* which have four pollinia (Christenson, 2001).

Group 2: Consisted of section *Proboscidioides* from genus *Phalaenopsis* and genus *Doritis*. The members of this group have four pollinia (Christenson, 2001).

Christenson (2001) indicated that *P. lobbii* and *D. pulcherrima* should be put in the same genus, *Phalaenopsis*, based on the high hybrid fertility between these two species. In this study, compatibility of cross between *D. pulcherrima* 'dwarf' and *P. equestris* showed close relationship between these two genera. In addition, Tsai *et al*., (2003) also proposed that *Doritis* and *Kingidium* should be treated as the genus *Phalaenopsis* by using molecular data from ITS 1 and ITS 2 sequences. Contrarily, molecular data from DNA sequences of the plastid genome, *matK* and *trnK* introns, and the nuclear genome, rDNA ITS, indicated that genera *Doritis* (section *Esmeralda*) and *Kingidium* (section *Deliciosae*) should be separated from genus *Phalaenopsis* (Tomohisa *et al*., 2005). However, Sweet (1980) separated these two genera, *Doritis*

and *Kingidium,* from genus *Phalaenopsis* based on the number of pollinia, the first two genera have four pollinia whereas most species in genus *Phalaenopsis* has two pollinia except for section *Parishianae* and *Proboscidioides*, have four pollinia. Even though, a few samples per species were tested, molecular data and crossability in this study also indicated that genera *Doritis* and *Kingidium* should be treated as genus *Phalaenopsis*.

5.2 Studies on crossability of genus *Phalaenopsis* and related genera, *Doritis* and *Kingidium*

Twenty-four interspecific crosses, i.e. 21 intersectional and 3 intrasectional, and 20 intergeneric crosses were made. From a total of 264 pollinations, 36 fruits were set in 24 crosses, which was only 13.64 % fruit setting. Fruit setting percentage of intersectional, intrasectional and intergeneric hybridizations were 14.07, 5.88 and 14.29 %, respectively. It was found that only 7 crosses could yield viable seeds which showed low crossability, 2.65 %, among the intersectional, intrasectional and intergeneric hybridizations. Other crosses, though with fruit setting, yielded no viable seed. Six-month old hybrid seedlings were transplanted from *in vitro* to 70 % shaded house condition. After six months, seedlings of the intersectional, intrasectional and intergeneric hybridizations showed a total of 74.58 % survival rate. Seedling of *P. schilleriana* x *P. cornu-cervi* showed the greatest survival rate, 81.25 %, whereas those of crosses *D. pulcherrima* 'dwarf' x *P. equestris*, *D. pulcherrima* 'dwarf' x *K. minus*, *P. violacea* x *P. javanica*, *P. amabilis* x *P. cornu-cervi*, *P. violacea* x *P. cornu-cervi* and *P. gibbosa* 1 x *P. parishii* yielded only 74.5, 70.8, 70, 64.29, 58 and 43.33 % survival rate, respectively.

Genetic distance values of all 44 interspecific and intergeneric crosses ranged from 0.15 to 0.67, however the 24 crosses that yielded fruit set had genetic distance values ranged from 0.15 to 0.58. The parents of 7 crosses that yield viable seed had close relationship 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 0.42, 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. These results were similar to reports from Wilfret and Kamemoto (1969) stated that failure in producing successful inter- and intrasectional crosses of *Dendrobium* showed some degree of relationship of species within the same section and between sections which indicated a close relationship. One of the main problems in making new hybrid cultivar was due to the difficulty of fruit setting.

Cross incompatibility is one of the problems needed to be solved in the *Phalaenopsis* breeding program. Compatibility is usually correlated to the closeness of their genetic relationship (Chen and Chen, 2007). RAPD analysis as shown by the previous study provided a rapid method to understand the genetic relationship of *Phalaenopsis* species and related genera, *Doritis* and *Kingidium*. In this study,

compatibility among *Phalaenopsis* species and two related genera was correlated with genetic similarity, it becomes a useful reference for selecting parental lines in orchid breeding program. In addition, the results of compatibility from intergeneric hybridization showed the close relationship between genus *Phalaenopsis* and 2 related genera, this result and genetic similarity analysis using RAPD technique supported that genera *Doritis* and *Kingidium* should be treated as genus *Phalaenopsis*.

Chromosome karyotype of the species corresponded with the crossability (Arends, 1970). All species of *Phalaenopsis*, *Doritis* and *Kingidium*, with the exception of the naturally occurring tetraploid *D. pulcherrima* var. *buyssoniana* (2n=4x=76), had the same chromosome number, 2n=2x=38 (Kao *et al*., 2001; Lin *et al*., 2001; Srithongroong, 1978), with chromosome sizes 1.5 - 3.5 μm (Arends, 1970). Arends (1970) studied on chromosome associations at metaphase I of 8 interspecific hybrids of *Phalaenopsis* and observed high frequencies of bivalents in hybrids between species both with large chromosomes (e.g. *P. amboinensis* x *P. mannii*) and between species both with small chromosomes (e.g. *P. amabilis* x *P. stuartiana*) but observed low frequencies of bivalents in hybrids between one species with large and one with small chromosomes (e.g. *P. mannii* x *P. equestris*). The results indicated that the similarity in karyotype of the species corresponded with their crossability. From an interesting study of fertility and crossability in *Phalaenopsis* and *Doritis*, Srithongroong (1978) found that the diploids and tetraploids had predominately normal tetrads and high seed fertility, both diploids and tetraploids could be crossed readily within and between the two genera. In this study, when reciprocal crosses were made of all compatible crosses, it was found that only few of them could produce hybrid. It showed that some species could be used as only female parent and

could not be used as pollen parent. The similar result was found in cross between *Vanda coerulea* and *Ascocentrum ampullaceum* var. *auranticum*, Kishor *et al*., (2006) stated that the crossability showed 60 % success of fruit development when *V. coerulea* was taken as female parent.

5.3 Characterizations of F_1 progenies derived from intersectional and intergeneric hybrids of *Phalaenopsis* and related genera, *Doritis* and *Kingidium*, by RAPD technique

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, however, their flowers had star-shaped similar to male parent and the flower colors showed transgressive segregation of the two parents. This hybrid was registered by Sukarya (1980) with the nomenclature *Phalaenopsis* Ayleen. Most progenies from crosses *D. pulcherrima* 'dwarf' x *P. equestris* and *D. pulcherrima* 'dwarf' x *K. minus* had dark green leaves, dark pink flowers and four pollinia which were similar to female parent while the flower size and shape were similar to male parent. Hybrid of cross *D. pulcherrima* 'dwarf' x *P. equestris* was registered by Twanaga (1963) with the nomenclature *Doritaenopsis* Purple Gem, and also hybrid of cross *D. pulcherrima* 'dwarf' x *K. minus* was registered by Liu (2002) with the nomenclature *Doridium* Sun Shia Swan.

RAPD technique was used to examine the relationship between parental lines and their 10 progenies. The 20 decamer primers were evaluated for amplification of 3 compatible crosses and their hybrids. 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 between *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. There were four primers, OPAK10, OPF01, OPF02 and OPF14, giving polymorphic DNA bands of all 3 compatible crosses and their progenies. These results were similar to the report by Inthawong *et al*. (2006) in hybrids of intersectional crosses derived from section *Phalaenanthe* x section *Formosae*, of *Dendrobium* which were evaluated using RAPD technique with 21 decamer primers. It was found that 7 primers, OPF01, OPF02, OPF03, OPF04, OPF05, OPF06 and OPD03, could yield good polymorphic pattern and confirm the intersectional hybrids. Inpar (2008) also stated that 4 primers, OPF01, OPF04, OPF07 and OPF10, could be used to identify relationship between parental lines and their progenies of cross between *Den.* Emma White x *Den. parishii*. Moreover, Minoo *et al*., (2006) reported that RAPD banding pattern of 4 interspecific hybrids of cross between *Vanilla planifolia* and *V. aphylla* displayed DNA band intermediate to their parents

In this study, hybrids showed genetic combination of their parents. RAPD profiles of hybrids when using suitable primers for each cross, clearly indicated that the DNA came from the two parents by the appearance of DNA markers, i.e. 1, 308 bp of OPD03 primer, 1,034 and 1,222 of OPF01 primer, 589, 731 and 1,500 bp of OPF02 primer, 740, 852 and 1,262 bp of OPF09 primer and 591, 748, 990 and 1,582 bp

of OPF14 primer in cross *P. schilleriana* x *P. cornu-cervi* , 295 and 1,123 bp of OPAK10 primer, 1,123 bp of OPD03 primer, 118 and 347 bp of OPF02 primer, 382 and 1,250 bp of OPF09 primer, 623, 700, 1,009, 1,210 and 1,579 bp of OPF14 primer in cross *D. pulcherrima* 'dwarf' x *K. minus* and 359 bp in cross *D. pulcherrima* 'dwarf' x *P. equestris*. The similar results were also reported in *Phalaenopsis* (Chen *et al*., 2001b) and in other orchids, such as *Cattleya* (Benner *et al*., 1995), *Dendrobium* (Inthawong *et al*., 2006 and Inpar, 2008) and *Vanilla* (Minoo *et al*., 2006).

There were some DNA bands of parents which were not present in the progenies. The numbers of absent DNA bands in progenies of *Phalaenopsis* were probably due to the segregation of heterozygous chromosomes during meiosis. According to the report of Huang *et al*. (2000) stated that losing some DNA bands from parents was caused by the segregation of heterozygous chromosomes and this loss was also found in the genetic analysis of chrysanthemum hybrids, based on RAPD technique. Smith *et al*. (1996) studied on interspecific hybridization among Hawaiian species of *Cyrtandra* using RAPD technique, and found that the RAPD profiles in hybrids were not completely additive of the patterns found in the parental species. DNA markers missing in the hybrids caused by chromosomal crossing-over during meiosis might have resulted in the loss of priming sites and thus RAPD markers of *Cyrtandra* species were presented in parents but not in progenies from interspecific crosses. William *et al*. (2000) also stated that variation in RAPD markers from parents to hybrids of soybean (*Glycine max* and *G. soja*) may have originated due to deletion, mutation, recombination or random segregation of the chromosomes at meiosis during the process of hybrid formation.

Genetic similarity between parental lines and their 10 progenies using principle component analysis (PCA) was performed 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. The similar result was found in the molecular characterization of 4 interspecific hybrids, VH1, VH4, VH5 and VH6, of cross between *Vanilla planifolia* and *V. aphylla* by RAPD marker. The results showed that VH1 and VH5 were clustered along with female parent, *V. planifolia*, while VH4 and VH6 were clustered along with male parent, *V. aphylla* (Minoo *et al*., 2006).

5.4 Specific marker for flower color pattern of *Phalaenopsis cornu-cervi* by AFLP technique.

During the selective amplification step, 64 primer combinations of 8 *Eco*RI and 8 *Mse*I primers: *Eco*RI+AC, *Eco*RI+AG, *Eco*RI+AAC, *Eco*RI+AAG, *Eco*RI+AGA, *Eco*RI+ATC, *Eco*RI+ATG, *Eco*RI+ATT, *Mse*I+CAA, *Mse*I+CAG, *Mse*I+CAT, *Mse*I+CCA, *Mse*I+CTA, *Mse*I+CTC, *Mse*I+CTG and *Mse*I+CTT, with 2 - 3 selective bases at 3' end were tested on 3 types of twelve *P. cornu-cervi*: 1) three plants of reddish brown flower; 2) six plants of yellow flower with reddish brown bars and spots; 3) three plants of pure yellow flower (no spot or bar). After

selective amplification step, agarose gel electrophoresis of plant No. 1 of *P. cornu-cervi* in reddish brown flower group was used to screen for suitable primer combinations. The results showed that the thirty primer combinations with polymorphic DNA bands and high resolution could be used to find the specific marker, which was found only in reddish brown flower and yellow flower with reddish brown bar and spot groups. Twelve *P. cornu-cervi* were reproducibly tested with thirty selected primer combinations. After selective amplification step, the denatured PCR products were separated on 6 % denaturing polyacrylamide gel electrophoresis. Four primer combinations, *Eco*RI+AC/*Mse*I+CAT, *Eco*RI+AG/ *Mse*I+CAG, *Eco*RI+AGA/*Mse*I+CAG and *Eco*RI+ATT/*Mse*I+CCA showed polymorphic DNA bands with high resolution and could be used to find the specific marker, which was found only in reddish brown flower and yellow flower with reddish brown bar and spot groups. The results showed 77 monomorphic DNA bands and 48 polymorphic DNA bands in the ranges of 180 - 2,800 bases. The *Eco*RI+AG/*Mse*I+CAG primer combination showed two specific DNA bands, which were found only in reddish brown flower and yellow flower with reddish brown bar and spot groups.

Two specific DNA bands were directly excised from dried polyacrylamide gel. The gel pieces were used to reamplify the fragment with the *Eco*RI+AG/ *Mse*I+CAG primer combinations using selective amplification condition. The fragments were then cloned with Clone JET^{TM} PCR Cloning Kit and automated sequencing (Ward Medic, Ltd.). The sequencing of two DNA fragments revealed 229 and 278 bases. DNA sequences were compared with DNA sequences database at National Center for Biotechnology Information (NCBI) GenBank. After BLAST

searches of the sequences, the 229- and 278-base sequences showed 78.7 % homology to the *Citrus reticulata* AFLP marker AFLP-4 genomic sequence which linked to the seedless trait (Xiao *et al*., 2009), and also the 229- and 278-base sequences showed 65.8 and 67.6 % homology, respectively, to the ATP synthase gamma chain mRNA of *Pyrus communis*. This protein is a member of important protein family and plays key roles during growth and development in various eukaryotes, such as produces ATP from ADP and regulating ATPase activity (Hiroyuki and Masasuke, 2001).

Two specific DNA markers in this study could assist to separate reddish brown flower and yellow flower with reddish brown spot or bar from pure yellow flower (no spot or bar) of *P. cornu-cervi*. However, after comparing result from the database, these two DNA bands did not show any matching with flower color or color pattern genes.

There was another evident that *Eco*RI+ATT/*Mse*I+CCA primer combination showed one specific DNA band at 670 bases, which was found in all 3 plants which have reddish brown flower and only 5 out of 6 plants which have yellow flower with reddish brown bar and spot groups. It indicated that losing DNA band in one plant of *P. cornu-cervi* was probably due to the DNA mutation or the DNA band was not gene that controlled any character of all plants.

In this study, two specific DNA markers of AFLP profiles from total genomic DNA were not linked to flower color related genes in the database. That might be due to the few samples of *P. cornu-cervi*, and the primers which were not suitable and could not amplify DNA fragment in the location of flower color related genes. This suggested in the future study, more samples of *P. cornu-cervi* and primers are required in order to find specific DNA markers of interest. Other specific molecular

techniques, such as cDNA-AFLP might be used to find specific DNA marker. The studies on molecular markers linked to flower color related genes were reported. Chen *et al*. (2001d) studied on gene expression of flower buds of two F_2 progenies derived from a cross between *P. equestris* "W9-52" and "W9-17" using cDNA-AFLP method. After analysis of the sequences, AM1-3 fragment showed 87 % amino acid sequence homology to the floral homeotic gene AGL5 of *Arabidopsis thaliana* and 90 % amino acid sequence to the gene AG of *Brassics napus*, which were transcription factors. AM4-1 fragment showed 66 % amino acid sequence homology to the gene GGPS6 of *A. thaliana*, which synthesized precursor of carotenoid. In addition, Chen *et al*. (2001a) using cDNA-AFLP compared the fingerprints of mRNA samples from the mature flower buds of *P.* Hsing Fei cv. H.F. and its somaclonal variant. Approximately 3,200 fragments were amplified after PCR with 32 primer combinations. Fifteen amplified fragments were specific for *P.* Hsing Fei cv. H.F., and the 12 fragments were specific for the variant. Sequence analysis showed that three of *P.* Hsing Fei cv. H.F. specific transcripts have 51 - 92 % homology to RNA - dependent RNA polymerase of *Cymbidium mosaic virus*. One variant-specific transcript showed 70 % identity in amino acid level to the mutator-like transposase of *Arabidopsis*. Hsu *et al*. (2008) further studied to confirm the differential gene expressions of these sequences using semi-quantitative RT-PCR. It was found that 5 sequences showed higher expression levels in the wild type plant compared to those in variant plant. These corresponded to sequences that encoded casein kinase, isocitrate dehydrogenase, cytochrome P450 and EMF2. These differential gene expressions may lead to the mosaic flower color and distorted lip morphogenesis of variant.

In this study, AFLP technique could provide markers that assisted separation of reddish brown flower and yellow flower with reddish brown spot or bar from pure yellow flower (no spot or bar) of *P. cornu-cervi*. The markers found in this experiment could be developed further for future use. Marker such as sequence characterized amplified region (SCAR) might be employed as an alternative method in order to confirm the result that these markers are marker-assisted selection of *Phalaenopsis* breeding program for flower color. Based on sequence information of cloned fragments, the two forward and reverse primers are designed and synthesized. SCAR primers are employed to amplify the genomic DNA to confirm the linkage of SCAR markers to the target trait. It is fast and efficient, and can assist breeders to determine the genetic background of material to ensure greater success in achieving the specific aims of hybridization.