CHAPTER 5

DISCUSSION

Habenaria and *Pecteilis* are terrestrial orchids. Each species has different floral characteristics. Both genera can be used as cut flowers and pot plants. They have attractive and beautiful flowers. These two genera share some common features such as flowers, tubers and leaves. Thus, molecular markers might be a good tool to provide information on genetics relationship of plant species. RAPD was chosen to detect some characteristics that could distinguish some species of these two genera.

5.1 Orchid morphology

Some morphological characteristics of some *Habenaria* and *Pecteilis* were alike and some were different. A total of six species and five varieties of *Habenaria* and *Pecteilis* were used in this study. The result of some characteristics have been described and grouped as follows:

5.1.1 Leaves

According to leaf arrangement, *Habenaria* and *Pecteilis* show some similarity i.e. *H. lindleyana* and *P. hawkesiana*, both have leaves cover ground. Leaves are thick and succulent. Others have spiral leaf arrangement which are thin and acute tip.

Leaf arrangement

Spiral leaf arrangement

- *H. rhodocheila* (orange, pink and red flower)
- *H. myriotricha*
- *H. xanthocheila*
- *P. susanae*

Cover – ground spiral leaf arrangement

- *H. lindleyana*
- *P. hawkesiana* (white and yellow lip)

5.1.2 Flowers

Flower color, there are two major of color groups, white and colors. *H. lindleyana*, *H. myriotricha*, *P. hawkesiana* and *P. susanae* have white flower whereas *H. rhodocheila* and *H. xanthocheila* have colored flowers.

Flower colors

White flower

- *H. lindleyana*
- *H. myriotricha*
- *H. xanthocheila*
- *P. hawkesiana* (white lip)
- *P. susanae*

* *P. hawkesiana* (yellow lip)

Colored flower

- *H. rhodocheila* (orange, pink and red flower)

Flower sizes

Less than 4 cm of flower width

- *H. rhodocheila* (orange, pink and red flower)
- *H. lindleyana*
- *H. myriotricha*
- *H. xanthocheila*
- *P. hawkesiana* (white and yellow lip)

More than 4 cm of flower width

- *P. susanae*

Results of plant morphology in this study aresomewhat similar to those that have been described by Kuanprasert (2005), Kurzweil (2009) and Kawchadee (2012). Batista *et al.* (2013) reported that most characters of *Habanaria* species were similar.

5. 2 Analysis of genetic relationship of genus *Habenaria* and *Pecteilis* by RAPD technique

5.2.1 DNA extraction

DNA extractions of *Habenaria* and *Pecteilis* were performed using four different extraction buffers, CTAB (Doyle and Doyle, 1990) and SDS (Ichiro *et al*., 2013) which was modified by adding 1% of PVPP to extraction buffer. DNA product could be obtained easier when SDS buffer added with 1% PVPP was used. CTAB buffer yielded DNA with a lot of oil contaminants, even 1% PVPP was added. The oily contaminants may be phenolic compound substances (Bennett and Wallsgrove, 1994). Plant cells are composed of polyphenol which may contaminate DNA extract. Polyphenols are eliminated by PVPP (Ichiro *et al*., 2013) which was presented in SDS buffer. It was easier for further pipetting steps. Quality and quantity of DNAs were determined by electrophoresis and spectrophotometer, respectively. DNAs absorb light at 260 nm. Absorbance of DNA, extracted by CTAB and SDS buffers, were 1.37 to 1.92 and 1.61 to 1.77, respectively. DNA quality was determined by agarose gel electrophoresis. The results showed that both CTAB and SDS buffers gave single DNA bands indicating that DNA sizes were of high molecular weight and DNAs did not break down. The smear may come from protein, polysaccharide, polyphenol and other substances.

5.2.2 Primer screening

To analyze genetic relationship between *Habenaria* and *Pecteils*, one hundred and forty primers, 20 each of OPA, OPC, OPD, OPF, OPG, OPN and OPU, were used. The results showed that some RAPD primers could amplify polymorphic bands in all samples while the others could not (Appendix C). Primer screening can help select the primer. Mao and Fang (2014) reported that 100 primers were screened for preliminary study of genetic diversity in *Haplocladium microphyllum* using ISSR. It was found that only 11 primers could provide clear and polymorphic bands. In another study, Huang *et al*. (2010) used 43 pairs of primers for analysis of diversity and relationship among Chinese orchid cultivars using EST-SSR markers. It was found that 13 primers could identify 103 Chinese orchids cultivars derived from six species.

5.2.3 Molecular marker analysis

Fifty-three primers could reveal polymorphism between *Habenaria* and *Pecteilis*. Specific bands were found by some primers**.**

OPD05$_{2,240}$, OPD13$_{1,543}$ and OPN10$_{1,591}$ could generate specific bands in *H. lindleyana* and *H. myritricha*.

OPA07$_{1,153}$, OPA16$_{858,}$ OPF01$_{959}$ and OPN05$_{605}$ could generate specific bands in *H. rhodocheila* (pink, orange and red flower).

OPD01$_{492,}$ OPD20$_{899,}$ OPD20$_{573}$ and OPU19$_{2,090}$ could generate specific bands in *P. hawkesiana* (white and yellow lip).

OPA10$_{362,}$ OPA20$_{864,}$ OPC04$_{786}$, OPC04$_{641,}$ OPC05$_{1,280,}$ OPD06 $_{295,}$ OPD08$_{1,194,}$ $_{790,}$ OPG07$_{580,}$ OPG10 $_{600,}$ OPG15$_{817,}$ OPN04$_{759}$ and OPN16 $_{910}$ could generate specific bands in *H. lindleyana*, *H. myritricha* and 2 species of *Pecteilis*.

OPA10$_{680,}$ OPA16$_{1,255,}$ OPA20$_{1,842,}$ $_{259,}$ OPC04$_{498,}$ OPC05$_{1,322,}$ OPC07$_{655,}$ OPC08$_{1,886,}$ OPD06$_{1,297,}$ OPD08 $_{534,}$ OPG04$_{1,209,}$ OPG04$_{903,}$ OPG09$_{735,}$ OPG13 $_{972,}$ OPG15$_{1,352,}$ OPG15$_{507,}$ OPG17$_{1,194,}$ OPN$_{1,151,}$ OPN20$_{646}$ and OPU15$_{1,315}$ could generate specific bands in *H.rhodocheila* and *H. xanthocheila*.

OPC16$_{1,268}$ and OPN05$_{268}$ could generate specific bands in *H. rhodocheila*, *H. xanthocheila* and 2 species of *Pecteilis*.

In this study, specific bands of *Habenaria* and *Pecteilis* were indicated by random primers. The specific bands can be developed as Sequenced Characterized Amplified Region Marker (SCAR) (Kaweewong, 2006). Manners *et al.* (2013) studied both RAPD and ISSR combined as SCAR in *Vanda coerulea* Griff ex Lindl (Blue Vanda). SCAR was used for analyzing intra and inters populations in genetic diversity of this orchid species to study breeding and genetic preservation. Sun and Wong (2011) developed ITS marker based on SCAR marker on *Paphiopedilum armeniacum*, *P. micranthum*, *P. delenatii* and their hybrids. The results showed three SCAR primers, SCAR-600armF/Pap-ITS2R, SCAR-300delF/Pap-ITS2R and SCAR-700micF/Pap-ITS2R. Band at 600 bp of *P. armeniacum* and its hybrid progenies were shown by SCAR-600armF/Pap-ITS2R primer. Band at 300 bp of *P. delenatii* and its hybrid

progenies were shown by SCAR-300delF/Pap-ITS2R primer and band at 700 bp of *P. micranthum* and its hybrid progenies were shown by SCAR-700micF/Pap-ITS2R primer. All of 3 markers could separate each species and theirs hybrid progenies. SCAR markers should be used for separating plant species and their hybrids.

5.2.4 Cluster analysis and principal coordinate analysis

The result from cluster analysis of genetic relationship by UPGMA and dendrogram showed that plants with colored flowers, *H. rhodocheila* and *H. xanthocheila*, were separated from plants with white flowers *H. lindleyana*, *H. myriotricha*, *P. hawkesiana* and *P. susannae*. This cluster implied that the primers used in this study might amplify genomic regions associated with the control of flower color. *Habenaria* and *Pecteilis* genetics are corresponded with morphological characteristics. *H. rhodocheila* and *H. xanthocheila* are different in flower colors but their leaf and flower shapes are similar (Piyatrakul, 2004). The combinations of primers in each primer set could not separate *H. lindleyana* and/ or *H. myriotricha* (data not shown) while fifteen chosen primers as OPA10, OPC05, OPC06, OPC07, OPC11, OPC14, OPC16, OPD05, OPD08, OPD12, OPG09, OPG15, OPN04, OPN05 and OPU12 could divide plant samples into 2 groups, as previously described. Besse *et al.* (2004) studied genetic relationship cultivated vanilla, *Vanilla planifolia*, *V. tahitensis* and *V. pompon*, using RAPD technique. It was found that three species of *Vanilla* had different origin. Eight RAPD primers could be used to identify the hybrids and genetic relationship, *V. planifolia* more closed to *V. tahitensis* than *V. pompon*. Okeyo and Kako (1997) used DNA from 36 *cymbidium* cultivars to identify cultivars. A total of 132 RAPD primers were used, 78% were polymorphic bands. Genetic distances among the cultivars ranged from 0.08–0.50 with an average of 0.29. Cluster analysis divided each other and parents with offsprings. Chung *et al*. (2005) studied genetic relationship between 21 *Paphiopedilum* and *Phragmipedium* species and 13 cultivars. It was found that both genera were divided into 2 groups. Group 1 included all *Paphiopedilum* species and 8 *Phragmipedium* species. Group 2 included *Phrag. Longiflorum* and Belle Hogue point'‘Bakara LeAnn', 'Mem. Dick Clements', 'Don Wimber' and 'Hanne, Popow', as *Phragmipedium* varieties.

Most dendrograms were not corresponded with PCoA because data recording methods were different. Banding patterns for UPGMA cluster analysis were recorded automatically by Gene tool program while PCoA was generated by GenAlEx 6.5 program which calculated genetic relationship from manually recorded banding patterns. Bands were recorded as absent (0) and present (1).

5.3 RAPD analysis in accordance with morphological characteristics

Dendrogram from the combinations of 15 primers including OPA10, OPC05, OPC06, OPC07, OPC11, OPC14, OPC16, OPD05, OPD08, OPD12, OPG09, OPG15, OPN04, OPN05 and OPU12 could distinguish flower color of *Habenaria* and *Pecteilis*. They could separate white flower from colored flower. A group of white color included *H. lindleyana*, *H. myriotricha*, *P. hawkesiana* and *P. susanae*, and a group of colored flower was *H. rhodocheila* and *H. xanthocheila*. The DNA results were corresponded with morphology. Tsai *et al.* (2002) also used random primers as OPB, OPC, OPE and OPM to find plant genetic relationship and identification of subtribe Oncidiinae genotypes. The results could separate 24 accessions into six groups and one individual group as Miltonia.

The combinations of chosen primers, however, could not separate a group of *Habenaria* from *Pecteilis.* Some primers could separate only *H. rhodocheila* and *H. xanthocheila* from *H. lindleyana*, *H. myritricha* and 2 species of *Pecteilis*. The combination of 15 primers could divide a group of *H. rhodocheila* and *H. xanthocheila* from a group of *H. lindleyana*, *H. myritricha* and 2 species of *Pecteilis*. The latter group could be divided into 2 subgroups as a subgroup of *H. lindleyana* and *H. myritricha* and a subgroup of *Pecteilis* (Figure 107).

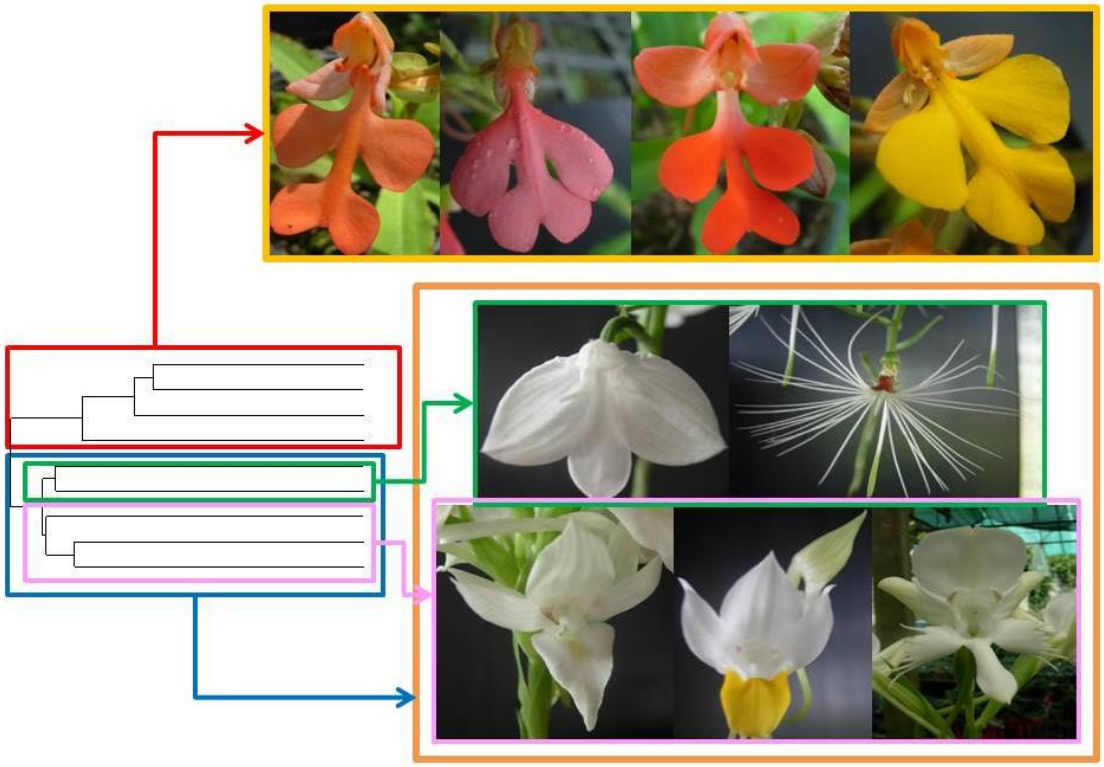

Figure 107 Dendrogram of *Habenaria* and *Pecteilis* based on 15 primers

Some primers could amplify specific bands. It was found that OPA10 primer could amplify specific marker as $OPA10_{362}$ marker could generate specific bands in *H. lindleyana*, *H. myritricha* and 2 species of *Pecteilis* and $OPA10_{680}$ could generate specific bands in *H. rhodocheila* and *H. xanthocheila*. These results corresponded to flower color morphology in which *H. rhodocheila* and *H. xanthocheila* show colored flowers and the others show white flowers.