CHAPTER 2

LITERATURE REVIEW

2.1 General information of genus *Phalaenopsis*

Phalaenopsis orchid is one of the most valued potted flowering plants in the world market, because its graceful flowers are long lasting both on plants and cut flower. *Phalaenopsis*, derived from Greek words, *Phalaina* meaning moth, -*opsis* meaning resembling, since its appearance of flowers is similar to some tropical moths (Sheehan and Sheehan, 1994). Its natural distribution is from India, through Southeast Asia to the Philippines, New Guinea and Australia (Sweet, 1980). Species of *Phalaenopsis* have a monopodial type of growth. Roots in *Phalaenopsis* are of three kinds: aerial, prostrate epiphytic and substrate. Plants of this genus all have short stems, leathery leaves up to 60 cm long and 15 - 20 cm wide (e.g. *P. gigantea*), shiny green or mottled with grayish green, often purple underneath. Growth habit of *Phalaenopsis* can be evergreen or deciduous as found in subgenera *Aphyllae*, *Parishianae* and *Proboscidioides*. The inflorescences or flower stalks range from short, few-flowered racemes to long-scapose racemes, to variously branched panicles. The rachis, or the flowering portion of the inflorescence, may be terete or variously flattened. A highly flattened rachis and floral bracts are characteristics of some species, such as those of the *P. cornu-cervi* complex. The flowers may be arranged in a spiral manner around the rachis (section *Stauroglottis*) or alternate, distichous forming a two ranked inflorescence (*P. bellina*). The rachis may be few to many flowered (Christenson, 2001).

The flowers are predominantly white, pink, yellow and mottled. Although they vary in size from 0.5 - 7.5 cm in diameter, the flowers are sufficiently alike to tie the genus together. The three sepals are alike in size and color. The two lateral petals are either much broader than the sepals (e.g. *P. amabilis*), equal to the sepals (e.g., *P. equestris*), or narrower than sepals in size (e.g. *P. pulchra*) and are usually of the same color. The lip varies considerably in this genus. The midlobe of the three-lobed lip may have two antennae or teeth at the apex. The lip also exhibits various appendages or callus-like tissue depending on the species. Some species have an entire midlobe on the lip. The column is short, narrow and often has the same color as the petals. The rostellum often has a pronounced beak in the center with an almost similar beak on the anther cap. Two round yellow pollinia are borne on an almost transparent stripe with a shield-shaped base (viscidium) that is very sticky. However, the species in subgenera *Aphyllae*, *Parishianae* and *Proboscidioides* all have four pollinia. The fruits of *Phalaenopsis* are green or variously pigmented. In particular, the fruits of subgenus *Aphyllae* are purple suffused and heavily mottled with dark purple-brown (Christenson, 2001).

All species of *Phalaenopsis* have the same chromosome numbers, 2n = 2x = 38, (Kao *et al*., 2001; Lin *et al*., 2001; Srithongroong, 1978) with chromosome sizes ranging from 1.5 - 3.5 μm (Arends, 1970). They can be divided into large, medium and small chromosome groups according to their chromosome size (Kao *et al*., 2001). By using flow cytometry, Lin *et al*. (2001) studied the nuclear DNA content of 18 species of *Phalaenopsis*. The quantities of the nuclear DNA content ranged from 2.74 - 16.61 pg/2C. They were classified into 8 groups according to the nuclear DNA content.

2.2 Classification of genus *Phalaenopsis*

According to Dressler's classification of the family Orchidaceae (Dressler, 1993), the genus *Phalaenopsis* Blume belongs to subfamily *Epidendroideae* Lindl., Tribe *Vandeae* Lindl. Subtribe *Aeridinae* Pfitzer. The history of classification of the genus has been reviewed by Sweet (1980), the genus *Phalaenopsis* comprises approximately 45 species that are grouped into 9 sections. In another revision, Christenson (2001) classified the genus *Phalaenopsis* into 5 subgenera from 65 species and treated the genera *Doritis* Lindl. and *Kingidium* P.F. Hunt as the genus *Phalaenopsis*, both of which have been treated as independent genera by other authors, e.g. Sweet (1980), Seidenfaden (1988) and Dressler (1993). This major inconsistency among taxonomists is largely due to different weighing attributed to the critical morphological character, pollinium number. Although pollinium number has traditionally been considered a heavily weighted character in classification of orchid genera, its state is not uniform within *Phalaenopsis.* The predominant number in the genus is two, except the sections *Aphyllae*, *Parishianae* and *Proboscidioides*, all have four pollinia, which are similar to the other genera *Doritis* and *Kingidium*.

Sweet (1980) divided genus *Phalaenopsis* into 9 sections and 4 subsections as follows:

Sweet' s classification (1980)

1. Section *Phalaenopsis*:

P. amabilis (L.) Blume., *P. intermedia* Lindl., *P. leucorrhoda* Rchb.f., *P. sanderana* Rchb.f., *P. schilleriana* Rchb.f., *P. stuartiana* Rchb f. and *P. veitchiana* Rchb.f.

2. Section *Proboscidioides*:

P. lowii Rchb.f.

3. Section *Aphyllae*:

P. stobartiana Rchb.f. and *P. wilsonii* Rolfe

4. Section *Parishianae*:

P. appendiculata C.E., *P. gibbosa* Sweet, *P. lobbii* (Rchb.f.) Sweet, *P. mysorensis* Saldanha and *P. parishii* Rchb.f.

5. Section *Polychilos*:

P. cornu-cervi Hassk., *P. lamelligera* Sweet, *P. mannii* Rchb.f., *P. pantherina* Rchb.f. and P. *valentinii* Rchb.f.

6. Section *Stauroglottis*:

P. celebensis Sweet, *P. equestris* (Schauer) Rchb.f. and *P. lindenii* Loher.

7. Section *Fuscatae*:

P. cochlearis Holttum, *P. fuscata* Rchb.f., *P. kunstleri* Hook.f. and *P. viridis* J.J. Smith.

8. Section *Amboinenses*:

P. amboinensis J.J. Smith, *P. gigantea* J.J. Smith, *P. javanica* J.J. Smith, *P. micholitzii* Rolfe and *P. robinsonii* J.J. Smith.

9. Section *Zebrinae*:

Subsection *Zebrinae*:

P. corningiana Rchb.f., *P. speciosa* Rchb.f. and *P. sumatrana* Korth & Rchb.f.

Subsection *Luddemannianae*:

P. fasciata Rchb.f., *P. fimbriata* J.J. Smith, *P. hieroglyphica* (Rchb.f.) Sweet, *P. lueddemanniana* Rchb.f., *P. pulchra* (Rchb.f.) Sweet, *P. reichenbachiana* Rchb.f. & Sander and *P. violacea* Witte.

Subsection *Hirsutae*:

P. mariae Burbidge ex Warner & Williams and *P. pallens* (Lindl.) Rchb.f.

Subsection *Glabrae*:

P. maculata Rchb.f. and *P. modesta* J.J. Smith.

Whereas Christenson (2001) combined genera *Doritis* and *Kingidium* into genus *Phalaenopsis* and reclassified these *Phalaenopsis* into 5 subgenera and 8 sections as follows:

Christenson's classification (2001)

1. Subgenus *Proboscidioides*:

P. lowii Rchb.f.

2. Subgenus *Aphyllae*:

P. braceana (J.D. Hook) E.A. Christ., *P. hainanensis* Tang & Wang, *P. honghenensis* F.Y. Liu, *P. minus* (Seidenf.) E.A. Christ., *P. stobartiana* Rchb.f., *P. taenialis* (Lindl.) E.A. Christ. & U.C. Pradhan and *P. wilsonii* Rolfe.

3. Subgenus *Parishianae*:

P. appendiculata C.E. Carr, *P. gibbosa* Sweet, *P. lobbii* (Rchb.f.) Sweet and *P. parishii* Rchb.f.

4. Subgenus *Polychilos*:

Section *Polychilos*:

P. borneensis Garay, *P. cornu-cervi* (Breda) Bl. & Rchb.f., *P. mannii* Rchb.f. and *P. pantherina* Rchb.f.

Section *Fuscatae*:

P. cochlearis Holtt., *P. fuscata* Rchb.f., *P. kunstleri* J.D. Hook. and *P. viridis* J.J. Smith.

Section *Amboinenses*:

P. amboinensis J.J. Smith, *P. bastianii* Gruss & Rollke, *P. bellina* (Rchb.f.) E.A. Christ., *P. deweryensis* Garay & E.A. Christ., *P. fasciata* Rchb.f., *P. fimbriata* J.J. Smith, *P. floresensis* Fowlie, *P. gersenii* (Teijsm. & Binn.) Rolfe, *P. gigantea* J.J. Smith, *P. hieroglyphica* (Rchb.f.) Sweet, *P. javanica* J.J. Smith, *P. lueddemanniana* Rchb.f., *P. luteola* Burbidge ex Garay, E.A. Christ. & Gruss, *P. maculata* Rchb.f., *P. mariae* Burbidge ex Warner & Williams, *P. micholitzii* Rolfe, *P. modesta* J.J. Smith, *P. pallens* (Lindl.) Rchb.f., *P. pulchra* (Rchb.f.) Sweet, *P. reichenbachiana* Rchb.f. & Sander, *P. robinsonii* J.J. Smith, *P. singuliflora* J.J. Smith, *P. venosa* Shim & Fowlie and *P. violacea* Witte.

Section *Zebrinae*:

P. corningiana Rchb.f., *P. inscriptiosinensis* Fowlie, *P. speciosa* Rchb.f., *P. sumatrana* Korth. & Rchb.f. and *P. tetraspis* Rchb.f.

5. Subgenus *Phalaenopsis*:

Section *Phalaenopsis*:

P. amabilis (L.) Blume, *P. aphrodite* Rchb.f., *P. intermedia* Lindl., *P. leucorrhoda* Rchb.f., *P. philippinensis* Golamco ex Fowlie & Tang, *P. sanderiana* Rchb.f., *P. schilleriana* Rchb.f., *P. stuartiana* Rchb.f. and *P. veitchiana* Rchb.f.

Section *Deliciosae*:

P. chibae Yukawa, *P. deliciosa* Rchb.f. and *P. mysorensis* Saldanha.

Section *Esmeralda*:

P. buyssoniana Rchb.f., *P. pulcherrima* (Lindl.) J.J. Smith and *P. regnieriana* Rchb.f.

Section *Stauroglottis*:

P. celebensis Sweet, *P. equestris* (Schaver) Rchb.f. and *P. lindenii* Loher.

2.3 Crossability in genus *Phalaenopsis*

Most of breeding programs have been done by the growers, thus, crossability of inter- and intra-section could not be revealed, even though, orchid breeding has been done since 1840. Crossability of orchid has been described in few genera. 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. Chen *et al.* (2000) studied the relationship between fertility and male or female parents used in the crosses, four varieties of *P. equestris* were used to cross with the commercial hybrids. The results showed that 50 - 57 % of the crosses produced viable seeds if *P. equestris* was used as male

parent. However, no viable seed was produced if *P. equestris* was used as female parent. The varieties of *P. equestris* used in this study were diploids while the other parents were tetraploid hybrids. That means failure of seed production was found when the tetraploid plants were used as male parents to cross with the diploid varieties. According to observations on the crossability between *Vanda coerulea* and *Ascocentrum ampullaceum* var. *auranticum* by Kishor *et al*. (2006), 60 % success with fruit development was found when *V. coerulea* was taken as female parent. The successful crosses developed fruits. However, the reciprocal cross with *A. ampullaceum* as female parent was not successful as there was no fruit development.

Chromosome karyotype of the species corresponded with their 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) observed the frequency of normal and irregular microspores in artificial F_1 hybrids of *Phalaenopsis*. The data reveal that intrasectional hybrids of sections *Phalaenopsis*, *Polychilos* and *Stauroglottis*, and other intersectional hybrids, i.e. *Phalaenopsis* x *Stauroglottis*, *Amboinenses* x *Polychilos* and *Esmeralda* x *Parishianae* had high percentages of normal microspores. Arends (1970) further studied chromosome associations at metaphase I of F_1 hybrids of *Phalaenopsis.*The result showed that intrasectional hybrids of sections *Phalaenopsis* and intersectional hybrids of *Phalaenopsis* x *Stauroglottis* and *Amboinenses* x *Polychilos* showed high levels of meiotic chromosome homology whereas intersectional hybrids, i.e.

Amboinenses x *Stauroglottis*, *Amboinenses* x *Phalaenopsis*, and *Polychilos* x *Stauroglottis* showed low levels of meiotic chromosome homology.

Crossability of *Dendrobium* has been intensively studied by Wilfret and Kamemoto (1969) made a systematic study utilizing 37 species of *Dendrobium* from 10 sections, out of 41 sections. They could make 721 pollinations, 138 fruits were harvested, and 89 produced viable seedling. Five intrasectional and 20 intersectional combinations resulted in successful hybrids. Later on, they made crosses among 8 horticultural important sections, and they could predict obtainable hybrid percentage and relationship among those observed species (Kamemoto and Wilfret, 1980). Furthermore, Kamemoto (1987) proposed the possibility of intersectional crosses from 4 sections, *Phalaenanthe*, *Ceratobium*, *Eleutheroglossum* and *Latourea*. This finding has given numerous impact on *Dendrobium* breeding program, especially for commercial potted cultivars.

2.4 Molecular markers for genetic analysis

Evolution, classification and variation of various species have been investigated by several different biological methods such as morphology, physiology and ecology. However, each method has several limitation. Systematic studies based on morphological analysis require a large number of samples and experienced scientists to decide whether investigated characters are informative. More importantly, some morphological characters are often environmentally influenced, therefore population of a particular species may be misclassified due to ecological variants. In recent years, after the development of molecular technology, the classical methods have been increasing by confirmed by molecular techniques, based on DNA polymorphisms (Weising *et al*., 1995).

Analysis of polymorphisms at the DNA level is the direct approach to study genetic variation at both inter- and intra-specific levels. There are various DNA-based techniques, however, each technique has different sensitivity of detection. To select the most appropriate technique for a particular problem, reasonable sensitivity, cost-effective, and time consuming are needed to be taken into account.

Molecular techniques based on DNA analysis

The morphological characteristics, cytology and isozyme analysis are generally used in the identification of new species and cultivars. However, these methods are limited by the environmental effects and the diagnostic resolution. Molecular marker techniques can help resolve relationships among plant materials (Xiang *et al*., 2003). DNA markers have been used to manipulate marker-assisted selection, and to guide the introgression of target genes from related species (Wolff *et al*., 1994). Early DNA-based techniques were mostly not applicable to conservation studies because the amount of DNA used required the destructive sampling of large amount of plant tissue. However, this situation changed dramatically with the invention of the polymerase chain reaction (PCR), which allowed the use of minute amount of DNA because it produced large numbers of copies of the fragments of DNA under study (Fay, 1999).

Polymerase chain reaction (PCR)

PCR has been invented since 1985 by Kary Mullis. It becomes a basic practice for advance molecular analysis. It is a technique which allows the amplification of a specific deoxyribonucleic acid (DNA) region that lies between two regions of known DNA sequence. PCR amplimers of DNA is achieved by using oligonucleotide

primers. These are short, single-stranded DNA molecules which are complementary to the ends of a defined sequence of DNA template. The primers are extended on single-stranded denatured DNA (template) by a DNA polymerase, in the presence of deoxynucleoside triphosphates (dNTPs) under suitable reaction conditions. The basic steps of PCR are (1) the denaturation by heating of template DNA to 94 - 98 °C for 20 - 30 sec, it causes DNA melting of the DNA template by disrupting the hydrogen bonds between complementary bases, yielding single strands of DNA, (2) the annealing of 10 - 14 nucleotide primers to the single strand DNA template at 50 - 65 °C for 20 - 40 sec and (3) the extension by DNA polymerase from the primers to copy the template DNA molecule at 72 °C for 120 - 240 sec. This results in the synthesis of new DNA strand complementary to the template strands. These strands exist at this stage as double stranded DNA molecules. Strand synthesis can be repeated by heat denaturation of the double-stranded DNA, annealing of primers by cooling the mixture and primer extension by DNA polymerase at a suitable temperature for the enzyme reaction. Each repetition of strand synthesis comprises a cycle of amplification. Each new DNA strand synthesized becomes a template for any further cycle of amplification and so the amplified target DNA sequence is selectively amplified cycle after cycle. The first extension product resulted from DNA polymerase will continue to synthesize new DNA until it either stops or is interrupted by the start of the next cycle. The second cycle extension products are also of indeterminate length; however, at the third cycle, fragments of target sequence are synthesized which are of defined length corresponding to the positions of the primers on the original template. From the fourth cycle onwards the target sequence is amplified exponentially. Thus, amplification as the final number of copies of the

target sequence is expressed by the formula, (2^n -2n) x, where; 'n' is the number of cycles and 'x' is the number of copies of original template (Newton and Graham, 1994; Karcher, 1995).

Restriction fragment length polymorphism (RFLP)

Restriction fragment length polymorphism (RFLP) is one of several techniques used to determine DNA variation based on the assumption that digested DNA fragments illustrating identical length are similar in sequences and they are from the same evolutionary origin. Technically, the target DNA digested with restriction endonucleases are size-fractionated by agarose gel electrophoresis and transferred onto a membrane. The investigated fragments are identified by hybridization with the specific radiolabeled probe (Davis *et al*., 1994). Smith and Smith (1991) used RFLPs in hybrid corn to confirm that it is a powerful tool in molecular systematics with numerous successful applications in crop species. However, RFLP is unpopular among scientists due to its technique is complicated and difficult as the technique requires radioactivity which is very dangerous (Auttatinpahorkun and Auttatinpahorkun, 1993).

The inheritance of chloroplasts was analyzed using RFLP technique in both interspecific hybrids of *Phalaenopsis* and intergeneric hybrids between *Phalaenopsis* and *Doritis*. Chloroplast DNA was digested with *Dra*I followed by hybridization with *rbc*L probe. The results revealed that *P. amabilis*, *P. aphrodite* and *P. stuartiana*, which belong to the section *Phalaenopsis*, and *P. equestris* had the same 2.0-kb fragment. Both *P. amboinensis* and *P. mannii* had a 2.3-kb fragment, while *D. pulcherrima* had a 3.5-kb fragment. In both interspecific and intergeneric hybrids, maternal inheritance of the chloroplast genome was detected. In the interspecific cross

between *P. amboinensis* and *P. amabilis*, all 5 F_1 progenies showed a maternal inheritance pattern of the chloroplast DNA by the presence of a 2.3-kb fragment derived from *P. amboinensis*, while all 5 F_1 progenies of the reciprocal cross showed 2.0-kb fragment derived from *P. amabilis*. In the intergeneric cross between *P. equestris* and *D. pulcherrima*, all 5 F_1 progenies showed a 2.0-kb fragment derived from *P. equestris*, while all 5 F_1 progenies of the reciprocal cross showed a 3.5-kb fragment derived from *D. pulcherrima*. The results suggested that the chloroplast DNA could be used as a marker for identification of parentship and phylogenetic studies of taxonomy (Chang *et al*., 2000). In addition, PCR-RFLP of ITS of rDNA was used to analyze genetic relationship of 25 *Dendrobium* species and 22 commercial hybrids. PCR-RFLP of ITS of rDNA with 6 enzymes revealed 41 DNA patterns of 23 *Dendrobium* species and 18 commercial hybrids. *Taq*I gave the most polymorphic pattern of 24 types. The results showed that 24 from 25 *Dendrobium* species and 18 from 22 commercial hybrids could be differentiated and ITS of rDNA had high polymorphism. Furthermore, genetic relationship of these *Dendrobium* samples was analyzed. It was found that *Dendrobium* species were clearly separated from commercial hybrids (Piyachokanakul and Huehne, 2005).

Amplified fragment length polymorphism (AFLP)

Amplified fragment length polymorphism (AFLP) is a technique for distinguishing closely related genotypes and specific DNA marker. This technique has been developed by Vos *et al*. (1995) through which selected restriction fragments from the digestion of total plant DNA are amplified by the polymerase chain reaction (PCR)**.** Genomic DNA is digested with the restriction enzymes, *Eco*RI and *Mse*I, and ligated with *Eco*RI and *Mse*I adapter. The product is amplified in the preselective

amplification step using primers with one selective base (*Eco*RI+N and *Mse*I+N primers). The selective amplification step is conducted with 2 - 3 selective bases at the 3' end of both primers. Molecular marker profiles based on AFLP can be used to detect variation at the DNA level and have proven to be extremely effective in distinguishing closely related genotypes. The advantages of this technique include reproducibility, high resolution, genome-wide distribution of markers and prior knowledge of genome being studied is not required. This technique has been used to analyze genetic relationship in *Phalaenopsis* (Chang *et al*., 2009; Chen *et al*., 2001a; Liu *et al*., 2003), *Dendrobium* (Xiang *et al*., 2003), *Aglaonema* (Chen *et al*., 2004a), *Alpinia* (Wongpunya, 2005), *Caladium* (Loh *et al.*, 1999), *Curcuma* (Ngoksamoe, 2003) and *Diffenbachia* (Chen *et al*., 2004b).

AFLP was employed to study on molecular markers linked to flower color related genes of *Phalaenopsis*. Chen *et al*. (2001a) 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. Two fragments AM1-3 and AM4-1 were found to be corresponded to flower color. 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 *Brassica 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*. (2001b) used cDNA-AFLP to compare the fingerprints of mRNA samples from the mature flower buds of *P.* Hsing Fei cv. H.F. and its somaclonal variant. Approximately 2,300 fragments were amplified after PCR with 32 primer combinations. Fifteen amplified fragments were

specific for *P.* Hsing Fei cv. H.F., and 12 fragments were specific for the variant. Sequence analysis showed that three of *P.* Hsing Fei cv. H.F. specific transcripts were 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.

Identification of AFLP markers linked to floral traits of *Dendrobium* progenies derived from cross *Den.* White Angel x (*Den.* Lucian Pink x *Den.* Black Spider) was conducted. Linkage was represented by occurrence rate contrast. It was found that 24 markers (occurrence contrast greater than 80 %) linked to number of inflorescences, number of flowers, inflorescence length, vase life, days to flower and flower color. In particular, 4 markers were found to be strongly (occurrence contrast greater than 80 %) linked to days to flower trait (Hong *et al*., 2000).

Moreover, AFLP technique was used to find specific markers linked to seedless trait in *Citrus reticulata.* After screening with 72 primer combinations, 5 AFLP markers were identified that putatively correlated with the target trait. The five fragments were cloned and sequenced, and BLAST searches showed that four of the markers had high homology to functional genes, providing some promising information that might have assisted in understanding the molecular mechanism of

seedlessness in citrus (Xiao *et al*., 2009). There was also a report that AFLP technique was employed to identify markers linked to seed coat color in *Brassica juncea*. Three AFLP markers were identified as being tightly linked to seed coat color trait and specific for brown seed (Negi *et al*., 2000).

Random amplified polymorphic DNA (RAPD)

Random amplified polymorphic DNA (RAPD) was developed by Williams *et al*. (1990) who demonstrated that genomic DNA from a distantly related group of organisms could be amplified using a single short primer (9 or 10 nucleotides long) composed of an arbitrary oligonucleotide sequence. The primer can be randomly designed without any prior knowledge of the sequence information of organisms under investigation. The only limitation is that the primers should have at least 50 % G+C content and should not contain palindromic sequences. Different random primers used with the same genomic DNA produce make the different numbers and sizes of DNA fragments (Ellsworth *et al*., 1993). After amplification, the amplified DNA patterns can be conveniently determined by agarose gel eletrophoresis. RAPD is particularly useful for species having limited genetic information or for organisms which have not been genetically investigated before. This technique has been recently shown to be an effective method in detecting polymorphism and thus is a powerful tool for species or cultivar identification. RAPD analysis also provides a rapid method to understand the phylogenetic relationship of different species. If this relationship is correlated with crossability among species, it becomes a useful reference for the choice of parents in the work of hybridization by the breeders (Chen and Chen, 2007).

RAPD technique has been employed to differentiate and evaluate quite a number of orchid genera and species. Twenty random primers, OPF01 - OPF20, were

used to analyze the RAPD patterns among five genera, five species in the genus *Phalaenopsis* and five clones in a species, *P. equestris*. Polymorphism was observed among them when a suitable primer was used in the PCR reaction. In this study, it was shown that 9, 8 and 3 primers produced considerable polymorphism which could distinguish among five genera, five species and five clones, respectively (Chen *et al*., 1995)

Fu *et al*. (1997) studied the relationship of 16 wild species of *Phalaenopsis* using RAPD markers. They found that the similarity coefficient and the relative order were stabilized when 20 primers were used to generate 381 DNA bands for analysis. By using the results of this analysis, 16 wild species of *Phalaenopsis* could be classified into five groups according to the similarity coefficient and the relative order as shown by the dendrogram. The authors claimed that 11 out of 16 species studied were matched between the grouping methods based on morphological characteristics and use of molecular markers. Furthermore, *P. amabilis* and *P. equestris* were the most closely related species according to the RAPD data, but they were classified into two far-related sections based on morphology. Similarly, *P. mannii* and *P. lueddemanniana* were considered to be closely related according to the RAPD data which was different from the traditional taxonomic classification.

Genetic relationship of 33 species of *Phalaenopsis* was studied to determine their genetic distances. Among 20 primers, five primers showed polymorphism. The dendrogram resulting from UPGMA cluster analysis separated the *Phalaenopsis* species into 8 groups (Been *et al*., 2001). The somaclonal variation in *Phalaenopsis* True Lady “B79-19” derived from tissue culture was studied, in 1,360 flowering somaclones, no apparent difference was found in the shape of leaves, whereas flowers

in some somaclones were deformed. The RAPD data indicated that normal and variant somaclones were not genetically identical (Chen *et al*., 1998). Moreover, there was also a report that RAPD technique was used to identify the markers linked to red floral trait which was controlled by a single dominant gene of *P. equestris*, white floral parent, red floral parent, F_1 progenies and F_2 progenies. A total of 920 primers were used for screening markers that were related to the red floral gene. It was found that a 380-bp DNA fragment (OPQ10-380) from OPQ10 primer linked to the red floral gene (Chen *et al*., 2001c).

Genetic relationship of *Phalaenopsis* hybrids and their parents was determined by RAPD technique using OPC07 primer. Among *P. amboinensis*, *P. amabilis*, F_1 progeny and three other wild species showed that the F_1 progeny can produce polymorphic bands derived from its parents that were different from other wild species (Chen *et al*., 2001b).

RAPD technique was also used in other orchid genera and species as followed. It was used to identify thirty-six *Cymbidium* cultivars. Ten primers were tested. It was found that 78 % of 132 samples presented clearly different polymorphic DNA bands, and *Cymbidium* Blue Smoke could be differentiated from other cultivars when OPA05 primer was employed (Obara-Okeyo and Kako, 1998). The interspecific and intraspecific relationships of 21 samples from 15 *Cymbidium* species were analyzed by using RAPD. Twenty-two primers were used in the analysis by comparing differences of DNA patterns of all species and cultivars. The results showed that 21 samples could be divided into two clusters based on ecological traits. One trait was temperature zone preference, with each *Cymbidium* preferring either an Asian or subtropical temperature zone. The group that comprised the subtropical *Cymbidium*

was *C. aloifolium*, *C. insigne* and *C. lowianum*. Another trait was basing on physiological and morphological characteristic. It was found that *C. lancifolium* and *C. aspidistrifolium* could be separated based on the different flowering physiology and unique leaf form. The groups identified by morphological, physiological and ecological characteristic were in full agreement with those determined by RAPD analysis (Choi *et al*., 2006).

Inthawong *et al*. (2006) reported that hybrids of intersectional crosses derived from section *Phalaenanthe* x section *Formosae* of *Dendrobium* were evaluated using RAPD technique with 21 decamer primers. It was found that 7 primers, OPD03, OPF01, OPF02, OPF03, OPF04, OPF05, and OPF06, 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 a cross between *Den.* Emma White x *Den. parishii*. Moreover, Minoo *et al*., (2006) reported that RAPD banding pattern of 4 interspecific hybrids of a cross between *Vanilla planifolia* and *V. aphylla* displayed DNA band intermediate to their parents.

The genetic diversity and relationships among twenty *Paphiopedilum* and fourteen *Phragmipedium* were determined by RAPD using 200 decamer primers, 100 primers of each UBC#2 and #7. It was found that UBC241 primers produced clearly distinguished *Paphiopedilum rothschildianum* from the other *Paphiopedilum* and *Phragmipedium sargentianum, Phrag. pearcei, Phrag. longifolium, Phrag.* Belle Hogue Point*, Phrag.* Bakara LeAn*, Phrag.* Mem. Dick Clements, *Phrag.* Don Wimber and *Phrag.* Hanne Popow (Chung *et al*., 2006). RAPD was used to analyze genetic relationship of some *Vanda* species. It was found that 8 primers, i.e. OPU3,

OPU5, OPU6, OPU7, OPU12, OPU13, OPU14 and OPU18 primers could separate strap-leaved from terete-leaved *Vanda* species. The results supported the suggestion that terete-leaved *V. teres* and *V. hookeriana* could be classified in the genus *Papilionanthe* and *V. sanderiana* should remain in the genus *Vanda* (Lim *et al*., 1999). In addition, RAPD technique was used to study the genetic variation associated with flower characteristics of *Rhynchostylis coelestis* and *R. gigantea*. It was found that 6 primers, i.e. OPAK01, OPAK11, OPD16, OPF10, OPF12 and OPF16 primers could be used to separate *R. coelestis* 'alba' from *R. coelestis*, whereas 4 primers, i.e. OPAK10, OPD05, OPD07 and OPF10 could be employed to distinguish *R. gigantea* 'red' from *R. gigantea* and *R. gigantea* 'alba' (Charoenpakdee and Bundithya, 2005).

RAPD technique was used to study the genetic relationship of other plants as followed. Genetic analysis of 10 species of *Curcuma* spp. was investigated at species and clonal level. Forty-eight random primers were used to amplify DNA fragments. Three primers, OPA20, OPD11 and OPAB04, produced 37 polymorphic DNA bands ranged in sizes from 200 to 1,700 bp. These DNA fingerprint patterns were able to distinguish and divide all the 10 species into two groups, early and medium flowering groups (Arunyawat, 1997). In addition, genetic relationship among 20 cultivars of litchi (*Litchi chinensis* Sonn.) was evaluated. Sixty-nine arbitrary primers were applied to amplify DNA products by PCR. It was found that 5 primers, OPAK10, OPAQ12, OPAS1O, OPB18 and OPC09, were able to produce 60 polymorphic DNA bands with sizes ranged from 200 to 2,000 bp. The results showed that 20 cultivars were classified into 2 major groups with each containing three sub-groups. This study indicated that RAPD was a powerful technique to distinguish *Litchi* cultivars (Chiangda, 1998).