

Chapter 1

Breeding and Development of New Varieties in *Phalaenopsis*

Ching-Yan Tang[†] and Wen-Huei Chen^{*,†}

One of the most important strategies to keep Taiwan as the leading producer of *Phalaenopsis* in the world, is breeding and development of new varieties. Pedigree analysis of the 12 most popular white hybrids of *Phalaenopsis* indicated that the tetraploids of *Phal. amabilis* and the hybrid, *Phal. Doris* were used frequently as parents of these hybrids. Besides the standard big flower *Phalaenopsis*, development of novelty varieties, such as the Harlequins and the multi-floral types constitute the new trends in the *Phalaenopsis* breeding programs and markets in the last decade. The somaclonal mutants of *Phal. Golden Peoker* and the wild species, *Phal. equestris* played an important role in the development of these novelty varieties. Breeding for new varieties of *Phalaenopsis* is lengthy and time consuming. New techniques are needed to increase the breeding efficiency of crops having long life cycles. The recent development of molecular markers, such as restricted fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD) and DNA amplification fingerprinting (DAF) and their applications in *Phalaenopsis* breeding are discussed and evaluated in this chapter.

1.1 Introduction

The 1980s was the decade that has divided the orchid business of Taiwan into two distinct phases. Before 1980, the cultivation of orchid

*Corresponding author.

[†]Department of Life Sciences, National University of Kaohsiung, Kaohsiung, Taiwan.

was considered a hobby. Most hobbyists were raising orchids in small scale with simple green-house facilities. With the rapid growth of economy, *Phalaenopsis* orchids became one of the most important commodity in the domestic as well as the international markets. Since 1988, Taiwan Sugar Corporation has started a comprehensive program to modernize *Phalaenopsis* production through intensive research effort, while more modern and well-equipped greenhouses¹ were built to meet the demands of an expanding market which could not be met through the activities of orchid hobbyists. Moreover, *Phalaenopsis* breeding became more professional and was usually well designed as compared with the trial and error approach of the traditional breeding programs.

Another change during this period was the product type in the export markets. Instead of cut flowers, the medium- and large-sized seedlings of selected hybrids became the major items for export. Consequently, *Phalaenopsis* growers in Taiwan had to equip themselves with modern greenhouses as well as facilities for mass production of hybrid seedlings which are normally derived from crosses of two high quality parental varieties. Seeds from the mature capsules are sown by *in vitro* method. Young seedlings developed in the test tube are transplanted into pots which are divided into four groups: small-, medium-, and large-sized seedlings and flowering plants, according to market demands.² Progeny test is used to evaluate and select the potential hybrids at different stages of development. To save time, parental plants used for hybridization to produce hybrids are propagated by the mericlone method, while evaluation of the new hybrids is in progress. Therefore, at the final stage of selection of the hybrids, there will be enough parental stocks available for making crosses to produce a large amount of hybrid seedlings for the market.³

The *Phalaenopsis* varieties used for breeding are usually divided into two groups — the standard big flower group and the novelty group. The standard big flower group includes the white, pink as well as the varieties with stripes, being derivatives of the white *Phal. amabilis* and the pink *Phal. schilleriana*. The varieties of the novelty group are usually small flowers with special coloration; some have special fragrances, i.e. if *Phal. violacea* is involved in the pedigree. Other parental varieties in this group are *Phal. amboinensis*, *Phal. venosa*, etc. In recent years,

the pot varieties which have small but plentiful flowers have become a new market trend. *Phal. equestris* and *Phal. stuartiana* are the common parental varieties of this group.

In general, the breeding programs are designed to improve the size and color of the flowers as well as other characteristics such as, longevity, stalk length, leaf shape, ease of cultivation, disease resistance and the number of viable seeds through the selection of parents for hybridization and so on. Through tremendous efforts in breeding, various types of *Phalaenopsis* varieties with attractive color and graceful appearance (Fig. 1.1) have been developed and the success of the development has made Taiwan one of the most important producers of *Phalaenopsis* in the world.

The growing cycles of *Phalaenopsis* orchids are long, a cycle being 2–3 years. Using the traditional hybridization to transmit useful traits into the commercial varieties is a long process which takes years to achieve. In addition, some species of orchids are cross-incompatible, thereby limiting the work of variety improvement. Hence, new approaches and techniques are needed in order to produce superior *Phalaenopsis* varieties for the fast growing and highly competitive markets. This chapter discusses the recent developments in the breeding work of *Phalaenopsis*.



Fig. 1.1. *Phalaenopsis* varieties showing various attractive colors and graceful appearance.

1.2 Development of *Phalaenopsis* Varieties by Hybridization

1.2.1 White *Phalaenopsis* varieties

The standard big white flower is the most important group of *Phalaenopsis* in the market (Fig. 1.2). Besides the large size, the breeding objectives of the white *Phalaenopsis* include long flower stalk, well-shaped flowers with long life span, etc. Taiwan is located at the northern border of the natural growth habitat of *Phalaenopsis*. A white flowered species, *Phal. amabilis* var. *formosa* was found native in Heng-Chung Peninsula, Taitung County and the Orchid Island off the coast of southern Taiwan.⁴ This native species had won several awards in different international orchid conferences as early as in the 1950s for the beauty of their multi-flowers. By using the technique of polyploidization, superior tetraploid varieties with short flower stalk, round-shaped petals and good quality flowers were developed. These varieties were well accepted by the Japanese market.

The modern superior large white hybrids were developed through the hybridization of breeding stocks from different sources, including those from the local *Phalaenopsis* farms and many from foreign countries, such as Japan, the Netherlands and the United States. Based on these materials, large, well-shaped white-flowered *Phalaenopsis*



Fig. 1.2. The appearance the standard big white variety “*Phal. Taisuco Brinasu*.”

hybrids with uniform morphology were developed. Through analysis of the pedigree of the 12 most popular white Taisuco *Phalaenopsis* hybrids in 1997/98, it was found that all of them were the offspring of *Phal. amabilis*, *Phal. rimestadiana*, *Phal. aphrodite*, *Phal. schilleriana*, *Phal. stuartiana* and *Phal. sanderiana* with the exception of the *Phal.* Taisuco white which was not related to *Phal. stuartiana* and *Phal. sanderiana* (Table 1.1). Among these wild species, *Phal. amabilis*, *Phal. rimestadiana* and *Phal. aphrodite* were the most important ancestors for the modern white commercial hybrids.⁵ The proportion of the genetic constitution contributed by *Phal. amabilis*, *Phal. rimestadiana* and *Phal. aphrodite* were 40.34%, 38.56%, and 16.41%, respectively. Based on this information, one can note that these hybrids were closely related in their genetic make-up. This narrow genetic background in *Phalaenopsis* white hybrids was difficult to avoid due to the demand for high uniformity of the hybrid seedlings by the market. That means genetic homogeneity of the parental stocks was required in order to produce uniform hybrid seedlings. However, one has to be aware that genetic depression may occur during the process of improvement.

From the same analysis, it was found that these 12 Taisuco hybrids were originated from 17 ancestral hybrids (Table 1.2). However, the

Table 1.1. Genetic Contribution of the Wild Species for the 12 Most Popular Commercial Hybrids^a of the White Taisuco *Phalaenopsis*

Wild Species Used in the Pedigree	Percentage of Genetic Contribution	Mean of Percentage	C.V. ^b
<i>Phal. amabilis</i>	39.11–42.19	40.34	2.8
<i>Phal. rimestadiana</i>	37.64–39.22	38.56	1.2
<i>Phal. aphrodite</i>	15.36–17.74	16.41	4.3
<i>Phal. schilleriana</i>	2.64–4.49	3.48	19.8
<i>Phal. stuartiana</i>	0–1.17	0.51	70.6
<i>Phal. sanderiana</i> ^c	0–0.78	0.47	51.1

^aThe name of the 12 hybrids are: *Phal.* Taisuco Kochdian, *Phal.* Taisuco Kaaladian, *Phal.* Taisuco Windian, *Phal.* Taisuco Bright, *Phal.* Taisuco Bridian, *Phal.* Taisuco Adian, *Phal.* Taisuco White, *Phal.* Taisuco Brinasu, *Phal.* Taisuco Silver, *Phal.* Taisuco Crane, *Phal.* Taisuco Swan, *Phal.* Taisuco Nasubula.

^bC.V. = coefficient of variation.

^cThe commercial hybrid, *Phal.* Taisuco White, does not have the genetic contribution of *Phal. stuartiana* and *Phal. sanderiana*.

Table 1.2. Genetic Contribution of the Important Parental Hybrids in the Pedigree of the 12 Most Popular Commercial Hybrids^a of the White Taisuco *Phalaenopsis*

Name of Parental Hybrid	Genetic Contribution (%)	No. of Commercial Hybrids
Group A		
<i>Phal.</i> Elisabethae	40.23	12
<i>Phal.</i> Gilles Gratiot	30.69	12
<i>Phal.</i> Katherine Siegart	31.56	12
Group B		
<i>Phal.</i> Doris	50.47	12
<i>Phal.</i> Doreen	17.36	9
<i>Phal.</i> La Canada	12.18	9
<i>Phal.</i> Winged Victory	19.37	12
Group C		
<i>Phal.</i> Grace Palm	23.57	12
<i>Phal.</i> Thomas Tucker	12.26	9
Group D		
<i>Phal.</i> Elinor Shaffer	18.75	10
<i>Phal.</i> Long Life	14.84	8
<i>Phal.</i> Opaline	14.84	8
<i>Phal.</i> Vallehigh	22.27	8
Group E		
<i>Phal.</i> Kochs Schneestern	27.78	9
<i>Phal.</i> Meridian	27.78	9
<i>Phal.</i> Mount Kaala	26.70	11
<i>Phal.</i> Schone Von Unna	14.84	8

^aThe names of the 12 hybrids are the same as in Table 1.1.

average genetic contribution for *Phal.* Doris to the current large white *Phalaenopsis* hybrids was about 50.47%, which was equally important to the direct parental hybrids. Through the genetic flow from the ancestors to the modern white Taisuco hybrids, it is observed that the superior clones of the large white Taisuco *Phalaenopsis* were developed firstly through the improvement of the genetic characters for *Phal.* Doris by chromosome doubling, resulting in a tetraploid with a larger genomic capacity to accumulate more additive alleles. Then it was

followed by backcrossing and hybridizing with its relatives to recombine and to accumulate desirable additive alleles for the flower size and other favorable traits. By this selection scheme, more than 30 Taisuco *Phalaenopsis* white hybrids were obtained and they won many awards throughout the world, including eight from the American Orchid Society.

1.2.2 *Harlequin (novelty) varieties*

Development of the Harlequin varieties is a new trend in *Phalaenopsis* breeding which was developed in Taiwan in the last 12 years. The most distinguished characteristic of this new group of *Phalaenopsis* is the appearance of large blotches of coalesced spots with intense color against the light creamy white or other colors. The blotching appears to be unstable. It may vary in size, shape and location from flower to flower. It was also found that temperature may influence the expression of the blotches.⁶ With cooler temperatures, the color intensity of the Harlequin spot will increase.

The breeding of the Harlequins began in Taiwan when the famous hybrid *Phal.* Golden Peoker “Brother” (*Phal.* Misty Green × *Phal.* Liu Tuen Shen, Reg. Brothers’s Orchid, 1983) was mericloned in the 1990s. The special feature of this variety is its creamy white flower with intense wine-colored spots. From the pedigree analysis, *Phal.* Golden Peoker was developed from 12 wild species through 11 generations.⁷ The genetic contribution in generating wine-colored spots is 25, 18.75, 12.5 and 6.25% from *Phal. gigantea*, *Phal. leuddemanniana*, *Phal. amboinensis* and *Phal. faciata*, respectively (Table 1.3). Besides the contribution of nicely spotted flowers in the particular group, these species also have a tendency to add other characters, such as leather-like texture, and flattened and round appearance of the flowers of the Harlequins. In the process of mericloneing the *Phal.* Golden Peoker “Brother,” somaclonal mutants with different, remarkably, fused spots of Harlequin pattern on the sepals and petals have been found in different orchid farms in Taiwan. Among these mutants, three of them, namely “Ever-spring,” “Nan Cho” and “S.J.” were most famous and received AOS recognition and awards. The mericlones of these mutants also produced flowers that were dominated with this Harlequin pattern (Fig. 1.3). From the emergence of these clones, intensive breeding for the Harlequins began. They were used to hybridize high quality

Table 1.3. Genetic Contribution of the Wild Species to *Phal.* Golden Peoker

Wild Species	No. of Times of Each Species Introduced into Each Generation											% of Genetic Contribution	
	1	2	3	4	5	6	7	8	9	10	11		
<i>P. gigantean</i>		1											25.00
<i>P. luddemanniana</i>			1	1									18.75
<i>P. rimestadiana</i>							4	19	18	9	2		15.04
<i>P. amboinensis</i>			1										12.50
<i>P. amabilis</i>							3	13	12	4			10.16
<i>P. aphrodite</i>							1	9	13	5	2		7.42
<i>P. faciata</i>				1									6.25
<i>P. sumarana</i>						1							1.56
<i>P. schilleriana</i>								2	2				1.27
<i>P. stuartiana</i>							1		1				1.07
<i>P. equestris</i>							1						0.78
<i>P. sanderiana</i>										1			0.20

Data from RHS98 (1998).



Fig. 1.3. Coalescence of red-brownish blotches on the flowers is the characteristic of Harlequin *Phalaenopsis* (*Phal.* Golden Peoker “A87-100”).

parental varieties possessing different flower colors and to create novel cultivars of Harlequin flowers.

Phal. Golden Peoker is an excellent parent. From 1992 to 2003, 423 hybrids developed from *Phal.* Golden Peoker were registered in Sander’s

list of orchid hybrids. Among them, 149 were the Harlequins. In addition to *Phal.* Golden Peoker, there were six important related varieties which were widely used in the breeding programs to create novel cultivars of Harlequins (Table 1.4). From 1994 to 2002, 58 Harlequin flowers won awards from the AOS. Among these varieties, *Phal.* Ever Spring Fairy “Tokai Silky Star” and *Dtps.* Chain Xen Diamond “Celebration” were so commanding that they received 90-points and won the much sought after FCC/AOS. Another variety, “*Dtps.* Ever Spring Prince” received seven AOS awards in 2001 and 2002, including two AMs and five HCCs.

Good progress has been made to breed for Harlequin varieties since the development of *Phal.* Golden Peoker “ES,” a somaclonal variant. Due to the fascinating and unpredictable pattern of the Harlequin flowers, there remains a lot of room for the improvement of this group of novelty variety in the future.

Table 1.4. Number of Registered Hybrids Derived from Harlequin Parents in the Breeding Program^a

Harlequin Variety	Parent	Generation	No. of Hybrids Registered
<i>Phal.</i> Golden Peoker ^b	<i>Phal.</i> Misty Green × <i>Phal.</i> Liu Tuen-Shen	0	96
<i>Phal.</i> Ever-spring King	<i>Phal.</i> Chih Shang’s Stripes × <i>Phal.</i> Golden Peoker	1	37
<i>Phal.</i> Ever-spring Light	<i>Phal.</i> Ever-spring Star × <i>Phal.</i> Golden Peoker	1	9
<i>Phal.</i> Ever-spring Prince	<i>Phal.</i> Golden Peoker × <i>Dtps.</i> Taisuco Beauty	1	8
<i>Phal.</i> Ching Her Prince	<i>Phal.</i> Ever-spring King × <i>Phal.</i> Golden Peoker	2	6
<i>Phal.</i> Haur Jin Diamond	<i>Phal.</i> Golden Peoker × <i>Phal.</i> Ching her Buddha	1	5
<i>Phal.</i> Ho’s Fantastic Splash	<i>Phal.</i> Ever-spring King × <i>Phal.</i> Ho’s French Fantasia	2	5

^aData from Wildcatt Orchids Database (2003) and RHS98 (1998).

^bNo. of crosses including all clones of *Phal.* Golden Peoker which have “Brother”, “Ever-spring”, “Nan-Cho”, “S.J.” and “BL”, etc.

1.2.3 Potted varieties

Before the 1980s, cut-flowers dominated the *Phalaenopsis* market. However, demands for potted *Phalaenopsis* varieties have increased tremendously in the last decade. Breeding for the potted-plant market is different from breeding for the cut-flower market. While the cut-flower market emphasized floral traits, for the potted-plant market, vegetative traits are equally important. These traits include small plants with considerable number of flowers, shortened and multiple branching of the inflorescence, easy growing and flowering, etc. At the beginning, potted varieties available for the market were usually smaller version of the standard *Phalaenopsis*. Selections were made for more compact growth and flowering. As the demand of potted varieties increased, special effort was made to develop hybrids for this sector of the market. For this purpose, *Phal. equestris* is being used as the most important parent in producing hybrids for potted varieties.⁸ *Phal. equestris* is a native species in the Philippines and is one of the two indigenous *Phalaenopsis* species in Taiwan. Bearing either white or pink flowers are two common forms of *Phal. equestris*. Sequential flowering having flowers all around the inflorescence and short flower stalks are special characteristics of this species. In addition to *Phal. equestris*, *Phal. stuartiana* and *Phal. schilleriana* are also important species used for breeding of potted varieties having heavily branching flowers. The first important hybrid in this line of breeding is *Phal. Cassandra* (*Phal. equestris* × *Phal. stuartiana*). Though it was made by Seden and was registered by Veitch in 1899,⁹ it was not heavily used in *Phalaenopsis* breeding until the 1960s. Numerous hybrids with multi-branching which is expected as a major characteristic of multi-floral varieties, were developed by using *Phal. Cassandra* as one of the parents in their pedigree. More than 150 first generation hybrids were registered using *Cassandra* as one of the parents. Recently, *Cassandra* hybrid was remade by crossing the tetraploid form of *Phal. equestris* “Riverbend” and *Phal. stuartiana*. The resulting hybrid was a triploid hybrid which was sterile. The tetraploid form of the other parental varieties is needed in order to make use of the advantages of tetraploid breeding. Breeding for potted *Phalaenopsis* varieties is a new trend in the markets. One can expect to see more and better varieties in the near future.

1.3 Breeding Behavior and Inheritance

1.3.1 Inheritance of floral color

Taiwan is one of the native habitats of *Phal. amabilis* and *Phal. equestris* which are used extensively in the development of *Phalaenopsis* hybrids.¹⁰ *Phal. equestris* which appeared naturally with pink or white flowers, produces branched inflorescences with a short juvenile period, and is naturally dwarf. It is an important parent for breeding the miniature type of plants which produce a large number of small flowers and are much easier to pack and to transport. It was also used to produce hybrids that had white petals and sepals with a red lip (semi-alba). Plants of *Phal. equestris* are highly variable in terms of morphology as well as in floral color. It can be divided into the following forms¹¹:

- (1) *Phal. equestris* var. *alba* — a pure white form; no yellow pigments on the callus.
- (2) *Phal. equestris* var. *aurea* — white flowers with solid yellow lip.
- (3) *Phal. equestris* var. *leucotante* — flowers with white lips and yellow callus.
- (4) *Phal. equestris* var. *rosea* — flowers with even red petals and sepals; color of the mid-lobe of the lip varies from deep red to light red.
- (5) *Phal. equestris* var. *leucaspis* — small flowers with white edges on pink petals and sepals; mid-lobe of the lip is purple or orange in color with white or yellow callus.

It was reported that several independent genes control the colors of the lip of *Phal. equestris* through the expression of both anthocyanins and carotenoids.¹² By crossing between the white and red forms of *Phal. equestris*, Fu *et al.*¹¹ reported that the pink floral color was controlled by a single dominant gene. This gene acts on the coloration of petals, sepals, the mid-lobe and the apex area of the side-lobe of the lip. Also, it is expressed as a brownish color through a pleiotrophic effect on the coloration of the floral stalk and the spot of the callus. Compared with the petals and sepals, the color inheritance of the lip is more complicated. Two dominant duplicated genes which are independent of the above mentioned red gene, control the yellow color of the base of the mid-lobe and side-lobe as well as the callus of the lip.

Since *Phal. equestris* is frequently used to cross with other hybrids, it has been found that the expression of these color-genes varies according to the different genetic backgrounds. For example, when a pink-flowered *Phal. equestris* was used to cross with various commercial hybrids of pink, yellow with magenta spots or semi-alba floral colors, the colors of the flower of the progenies were pink, orange with pink blush, lavender or white with pink splash, respectively. Retention of the pink color in the flowers of the progenies from crosses with different genetic backgrounds suggested that the inheritance of pink floral colors of *Phal. equestris* might be controlled by a dominant gene¹³ which was the same red gene as in the previous study. However, when the same *Phal. equestris* was used to cross with commercial hybrids of the white, orange or yellow varieties, flowers with pink lips and various degrees of pink blush were observed in the progenies. These results suggested the presence of two complementary genes, C and R which controlled the pink color in the flowers of *Phal. equestris*, similar to those in *Cattleya*.¹⁴

1.3.2 Influence of *Phalaenopsis equestris* parents on fertility

To study the relationship between fertility and pollen or pod parents used in the crosses, four varieties (including two white and two pink forms) of *Phal. equestris* were used to cross with the commercial hybrids. Each form was used as either pollen or pod parents and *vice versa* for the commercial hybrids. A total of 147 crosses were made and the fertility of each cross was determined by measuring the viable seeds produced from each cross. The results showed that 50–57% of the crosses (Table 1.5) produced viable seeds if the white or pink forms of *Phal. equestris* were used as pollen parents to cross with the commercial hybrids. However, no viable seed was produced if *Phal. equestris* was used as pod parents, regardless of the floral color. The varieties of *Phal. equestris* used in this study were diploids while the majority of the other parents were tetraploid hybrids. That means failure of seed production was found when the tetraploid plants were used as pollen parents to cross with the diploid varieties. Therefore, in order to enhance the breeding efficiency, a breeder has to use the diploid varieties as pollen parents if the counterparts are tetraploid plants.

Table 1.5. Effect of *Phalaenopsis equestris* Parents on the Fertility

Floral Color of <i>Phal. equestris</i>	<i>Phal. equestris</i> Used as Pod or Pollen Parents	Total No. of Crosses	Fertility > 0 ^a	
			No. of Crosses	% of Crosses
White	Pollen parent	36	18	50
White	Pod parent	10	0	0
Pink	Pollen parent	91	52	57
Pink	Pod parent	10	0	0

^aThe crosses with viable seeds were considered as fertility > 0.

1.4 Application of Molecular Markers in *Phalaenopsis* Breeding

1.4.1 Screening for red floral gene by RAPD markers

A single dominant gene was found to control the red floral color (as against white color) in *Phal. equestris*.¹¹ Due to the long life cycle of *Phalaenopsis* orchids, it is a time consuming procedure to identify the progenies carrying this gene after hybridization. Therefore, a rapid technique is needed for early detection of the presence of this gene in order to increase the efficiency of the breeding procedure. By using a stepwise screening method, Chen *et al.*¹⁵ identified a RAPD (random amplified polymorphic DNA) marker linked to the red floral gene in *Phal. equestris*. In this experiment, the leaf tissue of plants from the white and red parents, F1 and F2 progenies were subjected to RAPD analysis. In the first step, 920 primers were screened by RAPD analysis using the leaf-tissue from the white and red parents and a single F1 plant. One hundred and fifty (16.3%) of them were found to produce distinct DNA polymorphic bands in the red floral parent and F1 progeny. These were absent in the white floral parent (Table 1.6). In the second step, three F1 plants were used for the detection of polymorphic and homozygous bands that could distinguish the red floral parent and F1 progenies from the white floral parent. Homozygous trait could be confirmed if polymorphic bands were present in all three F1 plants. Among the 150 primers selected from the first step, 34 showed distinct polymorphic and homozygous DNA bands that could distinguish the red

Table 1.6. Numbers and Probabilities of Polymorphic Primers Detected by RAPD Analysis and PCR Reactions Required in the F1 and F2 Progenies of *Phalaenopsis equestris* from 920 Primers

	F1 Plants		F2 Plants
	First Screening	Second Screening	Third Screening
No. of polymorphic primers	150	34	1
Probability of polymorphism (%)	16.3	3.7	0.1
No. of PCR reactions	2,760	750	3,604

flowered parent and the three F1 progenies from the white flowered parent. The third screening was made by using 34 primers for 106 individual plants (84 from red, 22 from white) randomly selected from the F2 population. The results showed that the primer “OPQ-10” (5′-TGT-GCC-CGA-A-3′) generated a 380 bp DNA band (OPQ 10–380) that was linked to the red floral gene. Chi-square analysis indicated that the OPQ 10–380 marker and the red flower gene were two closely linked genes with a distance of 30.8 centiMorgan (cM) apart. With the use of this OPQ 10–380 marker, one can identify the presence of the red-flower gene in the progenies of hybridization at any stage of the plant development. In association with the *in situ* hybridization technique, the molecular marker may potentially be used for the identification and gene mapping of the chromosome where the red flower gene is located.

1.4.2 Investigation of the parental and phylogenetic relationship by RFLP markers in chloroplast DNA

Traditionally, morphological characteristics and cytological analysis were used for the classification of plant species as well as for the phylogenetic study. Recently, due to the fast development of biotechnology at the molecular level, molecular markers using restriction fragment length polymorphism (RELP) or random amplified polymorphic DNA (RAPD) were commonly used for various areas of plant sciences.^{16,17} Because of the specificity, consistency and precision of the performance of these molecular markers, these techniques became widely used to study the phylogenetic relationship of plant species or the parental relationship in the plant breeding programs.

In one of the studies, RFLP was used to analyze the mode of inheritance of chloroplasts in both interspecific hybrids of *Phalaenopsis* (*Phal. amabilis* × *Phal. amboinensis*; *Phal. mannii* × *Phal. stuartiana*) and intergeneric hybrids of *Phalaenopsis equestris* and *Doritis pulcherrima*.¹⁸ Chloroplast DNA digested with *Dra* I followed by hybridization with an *rbcL* probe revealed that *Phal. amabilis*, *Phal. aphrodite* and *Phal. stuartiana* had the same size 2.0-kb fragment while the *Phal. mannii* and *Phal. amboinensis* had a 2.3-kb fragment. The size of the fragment in *Doritis pulcherrima* was 3.5-kb. In the analysis of the interspecific reciprocal crosses between two *Phalaenopsis* species or the intergeneric reciprocal crosses between *Phal. equestris* and *Doritis pulcherrima*, similar results were found, i.e. the sizes of the fragments shown in the F1 progenies were the same as that in the maternal parents (Table 1.7). Therefore, maternal inheritance of the cpDNA as revealed by the RFLP markers was clearly demonstrated in the reciprocal crosses between the interspecific hybrids and intergeneric hybrids. These results suggested that cpDNA can be used as a marker for the identification of the parentage and for phylogenetic studies of taxonomy.

1.4.3 Use of RAPD markers for phylogenetic study and variety identification

By using the morphological characteristics of the petal and sepal, Sweet¹⁹ classified *Phalaenopsis* orchids into 45 species and 9 sections. All the species of *Phalaenopsis* have the same chromosome number ($2n = 38$) with chromosome sizes ranging from 1.5 to 3.5 μm .²⁰ They can be divided into large, medium and small chromosome groups according to their chromosome size.²¹ By using flow-cytometry, Lin *et al.*²²

Table 1.7. Polymorphism as Shown by the RFLP Markers in the Parental Lines and the F1 Progenies of the Interspecific Reciprocal Crosses of *Phalaenopsis*

Fragment Size (kb)	Parents		F1 Progenies	
	A ^a	B	A × B	B × A
2.3	+ ^b	–	+	–
2.0	–	+	–	+

^aA and B represent *Phal. amboinensis* and *Phal. amabilis*, respectively.

^b+ and – represent presence or absence of polymorphism as shown by RFLP markers.

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 eight groups according to the nuclear DNA content. This information is useful in terms of orchid classification as well as the phylogenetic relationship among species. In addition to the various approaches mentioned, RAPD analysis was also used for these purposes. Fu *et al.*²³ 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 (Table 1.8) according to the similarity

Table 1.8. Comparison of the Classifications of 16 Wild Species of *Phalaenopsis* According to the Dendrogram Generated by RAPD,²³ Morphological Characteristics¹⁹ and Chromosome Sizes²⁰

Species	Group According to RAPD Data ^a	Section According to Sweet, 1980	Group According to Chromosome Size
<i>Phal. micholitzii</i>	E	Amboinensis	NA ^b
<i>Phal. intermedia</i>	E	Phalaenopsis	NA
<i>Phal. manni</i>	D	Polychilos	Large
<i>Phal. lueddemanniana</i>	D	Zebrinae	Medium
<i>Phal. mariae</i>	D	Zebrinae	Small
<i>Phal. pulchra</i>	D	Zebrinae	NA
<i>Phal. sumatrana</i>	C	Zebrinae	NA
<i>Phal. venosa</i>	C	Amboinensis	Medium
<i>Phal. violacea</i>	C	Zebrinae	Large
<i>Phal. gigantea</i>	B	Amboinensis	Medium
<i>Phal. amboinensis</i>	B	Amboinensis	Medium
<i>Phal. schilleriana</i>	A	Phalaenopsis	Small
<i>Phal. stuartiana</i>	A	Phalaenopsis	Small
<i>Phal. amabilis</i>	A	Phalaenopsis	Small
<i>Phal. aphrodite</i>	A	Phalaenopsis	Small
<i>Phal. equestris</i>	A	Stauroglottis	Small

^aGrouping according to Fu *et al.*²³

^bNA = not available.

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, *Phal. amabilis* and *Phal. 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, *Phal. manni* and *Phal. lueddemanniana* were considered to be closely related according to the RAPD data which was different from the traditional taxonomic classification. If the cytogenetic evidence comes into the picture, one can find that the chromosome size of *Phal. amabilis* and *Phal. equestris* falls into to the small group, while those of the *Phal. manni* and *Phal. lueddemanniana* falls into to the large group. It is more reasonable to put the varieties having similar chromosome size into the same group as shown by the RAPD data, instead of into different groups as in the traditional classification based morphological characteristics. In addition, based on comparison between the dendrogram generated by the RAPD analysis and chromosome sizes as shown by the study of the karyotype,^{20,21,24} it is noted that the tendency is for the *Phalaenopsis* chromosome size to probably evolve from large to small, and its origin seems to be polyphyletic.

Cross-incompatibility is one of the problems needed to be solved in the *Phalaenopsis* breeding programs. Compatibility is usually correlated to the closeness of their phylogenetic relationship which is, on the other hand, related to the status of the chromosome (i.e. chromosome number and size) and the homology of the nuclear DNA. On the other hand, RAPD analysis as shown by the previous study provides a rapid method to understand the phylogenetic relationship of different species. If this relationship is correlated with compatibility among species, it becomes an useful reference for the choice of parents in the work of hybridization by the breeders.

The morphological characteristics, and cytological and isozyme analysis were generally used in the identification of new species and cultivars. However, these methods are limited by the environmental effects and the diagnostic resolution. Recently, DNA amplification fingerprinting (DAF) has been shown to be an effective method in detecting polymorphism and thus is a powerful tool for species or cultivar identification.²⁵ In a study, 20 random primers were used to

Table 1.9. DAF Patterns Generated by 20 Random Primers which could Distinguish among 5 Genera,^a 5 Species in *Phalaenopsis* and 5 Clones in *Phal. equestris*

Primer	Genera	Species	Clones	Primer	Genera	Species	Clones
OPF-1	– ^b	+	+	OPF-11	–	–	–
OPF-2	+	+	–	OPF-12	+	–	–
OPF-3	–	–	–	OPF-13	–	–	–
OPF-4	–	+	+	OPF-14	–	–	–
OPF-5	+	–	–	OPF-15	+	+	–
OPF-6	+	–	–	OPF-16	–	+	–
OPF-7	+	–	–	OPF-17	–	+	–
OPF-8	+	–	–	OPF-18	–	+	–
OPF-9	+	–	–	OPF-19	–	–	–
OPF-10	+	+	–	OPF-20	–	–	+

^a5 genera were: *Phalaenopsis*, *Doritis*, *Cattleya*, *Dendrobium*, *Cymbidium*; the 5 species of *Phalaenopsis* were: *Phal. amabilis*, *Phal. amboinensis*, *Phal. mannii*, *Phal. violacea*, *Phal. equestris*; 5 clones of *Phal. equestris*.

^b+ and – represent presence or absence of polymorphism as shown by RAPD markers.

analyze the DAF patterns among five genera, five species in the genus of *Phalaenopsis* and five clones in a species, *Phalaenopsis 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 (Table 1.9). Distinguishable bands of DAF patterns among the clones with similar genetic background were obtained when a suitable primer was used. Therefore, DAF is a powerful and useful tool to generate a group of molecular markers which represent the identity of a new variety. It is one of the means by which one can use to protect the patent rights of the new varieties in *Phalaenopsis* as well as in other species.

1.5 Conclusion and Prospective

The standard white *Phalaenopsis* is a successful “research and development” product from both the horticultural and industrial points of

view. Because of the development of the superior white *Phalaenopsis* varieties, not only did it lead to the opening of an international market for the *Phalaenopsis* business, but it also stimulated the modernization of the production facilities, technique and management for the orchid industry in Taiwan in the last two decades. In this review, one can find that the success of the standard white *Phalaenopsis* varieties was based on the discovery of the tetraploid from *Phal. amabilis* and the hybrid *Phal. Doris*. From the analysis of the 12 white TAISUCO varieties, these two tetraploids were involved in their pedigree one way or the other. This means the development of white *Phalaenopsis* is no longer at the diploid level; it is a kind of tetraploid breeding. Although there are only a few of tetraploid parental stocks, yet new and superior varieties of standard white *Phalaenopsis* varieties have been developed year after year. This indicates that the genetic heterogeneity of the tetraploid parents is broad enough to maintain the genetic variability for continuous selection. However, one cannot overlook the potential problem of genetic depression due to the narrow genetic background in these stocks. Exploration and development of new tetraploid breeding stocks with diverse genetic background is an urgent need in order to develop better white *Phalaenopsis* varieties as well as other types of moth orchid.

Development of the novelty varieties, including Harlequin and multifloral *Phalaenopsis* is a new trend in the orchid business since the last decade. It opens the door to the exploitation of the use of the genetic diversity in various wild species of *Phalaenopsis* to create new types of varieties besides the standard moth orchids. This approach in *Phalaenopsis* breeding including various kinds of interspecific and intergeneric crosses will form more diverse and unusual types of *Phalaenopsis* that may be important in the future market. Because of the creation of novelty varieties, demands for *Phalaenopsis* orchids should continue to grow in the future.

Phalaenopsis breeding is a lengthy and time consuming process due to the long life cycle. DNA markers associated with useful genes such as the red floral gene of *Phal. equestris* as reviewed in this chapter, will increase the breeding efficiency through identification of the desired offspring at the seedling stage. Use of the RFLP and RAPD markers to study the phylogenetic relationship of wild species or the parentage of breeding stocks will provide good information on the

genetic relationship among different species and cultivars. This kind of information is helpful for breeders to choose the parents for hybridization with more precision. The technique of DNA amplification fingerprinting (DAF) is useful to identify different varieties developed in a breeding program. With this method, a breeder can protect the patent rights of the *Phalaenopsis* hybrids produced.

Looking into the future, there will be unlimited opportunities for the expansion of *Phalaenopsis* orchid in the international markets. However, in order to maintain the competitiveness of Taiwan in the *Phalaenopsis* business, development of new and superior varieties is the key to success. Besides the traditional hybridization technique, effort on the exploration of new sources of genetic diversity as well as the development in the biotechnology of *Phalaenopsis* orchids to increase the breeding efficiency and accelerate the development of novelty varieties should be emphasized, so as to maintain the leading role of Taiwan in the international orchid business.

References

1. Huang TJ, Pan CL, Jean MH, Chen CF. (1996) Design and development of a greenhouse automatic environmental control system. Report of the Taiwan Sugar Research Institute **153**:53–74.
2. Chen WH, Wang YT. (1996) *Phalaenopsis* orchid culture. *Taiwan Sugar* **43**:11–16.
3. Chen WH, Chyou MS, Wu CC, *et al.* (1998) Breeding *Phalaenopsis*. Experimental Report of the Taiwan Sugar Research Institute **1997/1998**:94–102.
4. Lin TP. (1977) *Native Orchids in Taiwan*. Vol. 2. Chong Tao Company, Chiayi, Taiwan.
5. Chen WH, Chen YH, Chyou MS, *et al.* (1999) Development of white Taisuco *Phalaenopsis*. In: Clark J, Elliott WM, Tingley G, Biro J (eds.), *Proceedings of the 16th World Orchid Conference*. Vancouver, Canada, pp. 272–278.
6. Fighetti C. (2004) Passing the torch. *Phalaenopsis-J Int Phalaenopsis All*, Winter 2004:20–31.
7. Chen WH, Chen TC, Wu WL. (2004) The influence of *Phalaenopsis* Golden Peoker “Brother” on Harlequins. *Phalaenopsis-J Int Phalaenopsis All*, Summer 2004:14–16.
8. Harper T. (1991). Mutliflora *Phalaenopsis*: The contribution of *Phal. equestris* in breeding multifloras. *Amer Orch Soci Bull* **60**:106–114.

9. Griesbach RJ. (2002) Development of *Phalaenopsis* orchids for the mass-market. In: Janick J, Whipkey A (eds.), *Trends in New Crops and New Uses*. ASHS Press, Alexandria, VA.
10. Stubbings J. (2006) Development of white with colored lip *Phalaenopsis*. In: Hwang JH (ed.), *Proceedings of Taiwan International Orchid Symposium*, Taiwan Orchid Growers Association, Tainan, Taiwan, pp. 38–51.
11. Fu YM, Chen WH, Tsai WT *et al.* (1996) Studies on floral color heredity of *Phalaenopsis equestris*. Report of the Taiwan Sugar Research Institute **152**:35–49. (In Chinese with English abstract).
12. Christense EA. (2001) *Phalaenopsis: A Monograph*. Timber Press, Inc., Portland, Oregon.
13. Chen WH, Tsai WT, Chyou MS, *et al.* (2000) The breeding behavior of *Phalaenopsis equestris* (Schauer) Rchb.f. *Taiwan Sugar* **47**(1):11–14.
14. Lenz LW, Wimber DE. (1959) Hybridization and inheritance in orchids. In: Wither CL (ed.), *The Orchids: A Scientific Survey*. Ronald Press, New York, pp. 261–314.
15. Chen WH, Fu YM, Lin YS, Chen YH. (2001) Identification of RAPD markers linked to the red floral gene in *Phalaenopsis equestris* by a stepwise screening method. *Taiwan Sugar* **48**(4):23–29.
16. Williams JGK, Kubelik AR, Livake KJ, *et al.* (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucl Acids Res* **18**:6532–6535.
17. Paran I, Kesseli R, Michelmore R. (1991) Identification of restriction fragment length polymorphism and random amplification polymorphic DNA markers linked to downy mildew resistance genes in lettuce using near-isogenic line. *Genome* **34**:1021–1027.
18. Chang SB, Chen WH, Chen HH, *et al.* (2000) RFLP and inheritance patterns of chloroplast DNA in intergeneric hybrids of *Phalaenopsis* and *Doritis*. *Bot Bull Acad Sin* **41**:219–223.
19. Sweet HR. (1980) The Genus *Phalaenopsis*. *The Orchid Digest*, Inc. USA.
20. Arends JC. (1970) Cytological observation on genome homology in eight interspecific hybrids of *Phalaenopsis*. *Genetica* **41**:88–100.
21. Shindo K, Kamemoto H. (1963) Karyotype analysis of some species of *Phalaenopsis*. *Cytologia* **28**:390–398.
22. Lin S, Lee HC, Chen WH, *et al.* (2001) Nuclear DNA contents of *Phalaenopsis* species and *Doritis pulcherrima*. *J Amer Soc Hort Sci* **126**(2):195–199.
23. Fu YM, Chen WH, Tsai WT, *et al.* (1997) Phylogentic studies of taxonomy and evolution among wild species of *Phalaenopsis* by random amplified polymorphic DNA markers. Report of the Taiwan Sugar Research Institute **157**:27–42. (In Chinese with English abstract).

24. Sagawa Y. (1962) Cytological studies of the genus *Phalaenopsis*. *Amer Orchid Bul* **31**:459–465.
25. Chen WH, Fu YM, Hsieh RM, *et al.* (1995) Application of DNA amplification fingerprinting in the breeding of *Phalaenopsis* orchid. In: Terzi M, *et al.* (eds.), *Current Issues in Plant Molecular and Cellular Biology*. Kluwer Academic, Netherlands, pp. 341–346.