A Genetic Linkage Map of Phalaenopsis-based on AFLP Markers and the “Two-way Pseudo-testcross” Mapping Strategy

Shenping Xu¹² and Feixiong Liao*²
¹Institute of Bioengineering, Zhengzhou Normal University, Zhengzhou 450044, China
²College of Forestry and Landscape Architecture, South China Agricultural University, Guangzhou 510642, China
*For correspondence: fxliao@scau.edu.cn

Abstract

Phalaenopsis are grown widely as cut-flowers and potted plants in the floriculture trade. Here, we report the genetic linkage maps for Phalaenopsis, which were developed by genotyping 88 F₁ progenies from a cross between Phalaenopsis ‘462’ (male) and Phalaenopsis ‘20’ (female) with amplification fragment length polymorphisms (AFLP) in a “two-way pseudo-testcross” mapping strategy. For a total of 416 polymorphic loci showing Mendelian segregation were generated from 71 AFLP primer combinations. The data were analyzed using JoinMap 3.0 to construct the parent-specific linkage maps. The paternal map consisted of 15 linkage groups and 122 AFLP makers, covering 820.3 cM with a mean distance of 6.7 cM between adjacent markers. The maternal map consisted of 14 linkage groups with 175 loci, resulting in a total genetic distance of 878.3 cM and an average genetic distance of 5.0 cM between adjacent markers. Yet, as far as we know, this is the first study to construct the linkage map of Phalaenopsis. The two maps will serve as the frameworks for mapping of horticultural quantitative trait loci (QTLs) and furnish reference information for the application of future marker-assisted selection in Phalaenopsis breeding. © 2017 Friends Science Publishers

Keywords: Orchid; Molecular marker; Genetic analysis; Linkage

Introduction

The genus Phalaenopsis is one of the most commercially important orchids in the family Orchidaceae, grown as cut flowers and potted plants due to their long-lasting flowers and graceful appearance. Phalaenopsis industry is growing around the world in recent decade. Numerous varieties of Phalaenopsis have been developed and registered with the Royal Horticulture Society (RHS) each year. However, very limited research efforts have been devoted to understanding the genetic diversity (Been et al., 2002; Chang et al., 2009) or the genomic features of Phalaenopsis (Hsu et al., 2008) and genetic transformation (Liao et al., 2004; Sjahril et al., 2006). Although one Phalaenopsis genome was recently sequenced (Cai et al., 2015), the inheritance of important ornamental or horticultural traits, genetic relationships among important species, and the genomic organization of Phalaenopsis remained poorly understood, due to the complex genetic background and extensive hybridization in this genus.

Genetic linkage maps provide a powerful tool for comprehending the genetic basis of sophisticated traits in many plants (Lee, 1995). Particularly they are very useful for map-based cloning and the localization of important genes and assisting plant breeders in the selection of breeding parents and breeding lines. Genetic mapping in plants are often constructed utilizing segregating populations from crosses between inbred lines, but this strategy is not possible for many of those plants that have high heterozygosity and long life cycles, such as forest trees (Grattaglia and Sederoff, 1994), fruit trees (Kenis and Keulemans, 2005), perennial flowers (Dunemann et al., 1999; Hibrand-Saint Oyant et al., 2008), etc. For these plants, the strategy of “two-way pseudo-testcross” has been adopted frequently. With this strategy, genetic linkage maps are constructed utilizing F₁ offspring derived from the cross of two highly heterozygous individuals (Grattaglia and Sederoff, 1994). For example, the method has been utilized successfully to construct linkage maps in agronomic crops such as peanut (Hong et al., 2008) and sweetpotato (Kriegner et al., 2003), ornamental plants such as wintersweet (Chen and Chen, 2010) and dendrobium (Xue et al., 2010), and tree species such as pistacia (Turkeli and Kafkas, 2013) and Eucamnia ulmoides (Wang et al., 2014).

Many species in Phalaenopsis are diploids with a chromosome number of 2n = 2x = 38, although some species have different chromosome numbers as revealed by cytological studies (Christenson, 2001; Kao et al., 2001). Hybridization occurs easily in Phalaenopsis, not only between the species but also with members of related genera. Because of frequent hybridizations, most Phalaenopsis varieties are highly heterozygous. In addition,
developing inbred lines in *Phalaenopsis* has been rather difficult. Considering these factors, the “two-way pseudo-testcross” strategy seemed to be an effective and convenient way for constructing genetic linkage maps in *Phalaenopsis*. The objective of this study was to apply the AFLP marker system to *Phalaenopsis*, to develop maternal and paternal pure-coupling phase AFLP markers, and construct linkage maps using the mapping strategy of “two-way pseudo-testcross”. We expect that these markers and the linkage maps would be valuable for identifying markers associated with important traits of interest and improving the breeding efficiency in this important orchid.

**Materials and Methods**

**Plant Materials**

The mapping population used was a hybrid population developed from two cultivars, *Phalaenopsis ‘20’* and ‘462’. *Phalaenopsis ‘20’* was characterized by creamy yellow flowers with small mahogany spots, medium-sized leaves, and slight fragrance, and it was used as the female parent of the population. The male parent was *Phalaenopsis ‘462’*, whose flowers were creamy white overlaid with large waxy magenta spots. This variety had large leaves and no fragrance. Hybrid seedlings were produced by germinating the seeds on an aseptic MS medium with 30 g·L\(^{-1}\) sucrose at 30°C for 6 months. A stock of hybrid seedlings was developed in an aseptic greenhouse for 6 months. A hybrid was produced by germinating seedlings on an aseptic MS medium with 30 g·L\(^{-1}\) sucrose at 30°C for 30 min. During the incubation, samples were shaken gently. The extract was mixed with an equal volume of octanol:chloroform (1:24), the aqueous phase was transferred to a clean centrifuge tube after centrifugation (13,000 × g, 10 min). DNA was precipitated from the supernatant by adding absolute alcohol and centrifugation. The pellet of DNA was washed with 75% alcohol and finally dissolved in 100 μL of TE buffer and incubated at 37°C for 1 h. The quantity and quality of the DNA were appraised by electrophoresis on 1% Agarose-gels with a standard weight Lambda DNA (Sigma).

**AFLP Protocol, Primer Screening and Marker Scoring**

AFLPs analyses were performed using the restriction enzyme combination *PstI* and *MseI* according to the method of Vos et al. (1995). For each progeny, 100 ng of genomic DNA was digested in a 12.5 μL reaction with 2.5 units of *PstI* and *MseI* (Sigma) at 37°C for 6 h. The reaction contained 1.25 μL of 10 × NE buffer 4 and 0.15 μL of 100 × BSA. After digestion, 1.5 μL of 10 × T4 DNA ligase buffer, 1.25 units of T4 DNA ligase and 1.25 units of *PstI* and *MseI* adapters were added to the reaction and the reaction was incubated at 37°C for another 3 h to ligate the adapters to the restricted DNA fragments.

After enzymatic restriction and adaptor ligation, an aliquot of 2.5 μL of the reaction was diluted 60 times with TE Buffer, and the diluted reaction was used for pre-amplification. The PCR reaction for the pre-amplification contained, 35 ng of *PstI* and *MseI* primers, 0.5 μL of dNTP mixture (10 mM), 0.5 μL of MgCl\(_2\) stock solution (25 mM), 0.9 μL of 10 × PCR buffer (non-Mg\(^{2+}\)), 5 units of Taq DNA Polymerase in a total volume of 25 μL. The pre-amplification PCR was performed on PCR instrument (Eppendorf, Hamburg, Germany) with the cycling program of described by Vos et al. (1995).

All pre-amplification reactions were diluted 60 times with purified water and then 1 μL of this dilution was used in a 20 μL volume mixture for selective amplification. Each selective amplification reaction contained 5 ng of the *PstI* primer, 30 ng of the *MseI* primer, 0.4 μL of dNTP mixture (10 mM), 2 μL of the 10 × PCR buffer (non-Mg\(^{2+}\)), 1.6 μL of MgCl\(_2\) solution (25 mM), and 0.4 units of Taq DNA Polymerase. Then the sample were amplified by PCR system and separated by 6% denatured polyacrylamide gels and DNA bands were visualized by silver staining. Primer combinations with three selective nucleotides (*MseI*-I+3/*PstI*-3) were screened to identify those that could amplify clear and unambiguous polymorphic fragments in both *Phalaenopsis ‘462’* and ‘20’.

AFLP marker fragments on the silver stained gels were manually scored for their absence (0) or presence (1). The distinguished clearly fragments were scored and recorded. There were a few ambiguous fragments in a few individuals. These fragments were resolved by assigning a blank score (−) for map construction. All AFLP markers were identified by primer combinations utilizing the primer notation (Vos et al., 1995). The bands of polymorphic were named serially in the descending order of molecular weight. Recorded markers would be split into three groups according to the absence or presence within each parent. AFLP fragments that were present exclusively in the male parent were given the prefix M, while those present only in the female parent were given the prefix F, and those fragments appearing in both parents were given the prefix MF.

**Data Analysis and Genetic Linkage Map Construction**

In the pseudo-testcross configuration, Mendelian segregation was tested for all markers at a 5%
signification level by performing a chi square ($\chi^2$) goodness-of-fit test to testcross (1:1) and intercross (3:1) marker ratios. The segregation of markers heterozygous in one parent was tested against a 1:1 ratio using a $\chi^2$ test, while those heterozygous in two parents were tested against a ratio of 3:1. Those markers that did not segregate in the 1:1 or 3:1 ratio were treated as distorted ones.

AFLP markers were categorized into three types: (1) those manifesting segregation only in the male parent (*Phalaenopsis* ‘462’), (2) those manifesting segregation only in the female parent (*Phalaenopsis* ‘20’), and (3) those manifesting segregation in two parents. AFLP markers of the three types were used to construct the genetic linkage map for *Phalaenopsis*, and types 1, 3 for *Phalaenopsis* ‘462’, types 2 and 3 for *Phalaenopsis* ‘20’, following the methods of Grattapaglia and Sederoff (1994). Linkage maps were generated independently for each parent using JoinMap 3.0 (Van Ooijen and Voorrips, 2001) based on LOD scores (minimum LOD score of 3.0). The detection of linkage among AFLP markers were based on pair-wise recombination estimates with a threshold recombination fraction $< 0.45$ and a threshold LOD score $> 3.0$. Genetic distances were converted from recombination fractions into centiMorgans (cM) according to Kosambi (1943). Final linkage mapping was drawn using the software MapChart 2.1 (Voorrips, 2002).

**Results**

**Polymorphism of AFLP Markers**

Out of the 570 AFLP primer combinations tested, 71 showed polymorphisms that were highly reproducible and then used to evaluate the 88 F$_1$ progenies. Through the analysis of AFLP, the fragments size ranged from 50 to 1500 bp. These 71 primer combinations produced 865 amplification products, of which 416 showed polymorphic characteristics (Table 1). The number of polymorphic AFLP markers generated by each primer combination varied from 1 to 13, with an average of 5.86 polymorphic fragments per primer combination. Of these AFLP markers, 149 bands were amplified in the male parent and 226 in the female parent, and these markers were expected to segregate in a 1:1 ratio ($P < 0.05$). Forty-one markers were detected in two parents and they were predicted to segregate in a 3:1 ratio ($P < 0.05$) in the F$_1$ population.

Though the analysis of chi square, the 314 (75.48%) polymorphic markers showed a compatible fit to the anticipated segregation ratio of 1:1 or 3:1, and 102 (24.52%) polymorphic markers gave a significant segregation distortion from the anticipated ratio of 1:1 or 3:1 ($P = 0.05$). Out of 314 polymorphic markers, 175 (55.73%) were segregating in female, 109 (34.71%) through male and 30 (9.56%) through co-parental.

**Map Construction**

When the 416 AFLP markers were suffered to two-point linkage analysis using JoinMap 3.0, 292 markers were found suitable for linkage map construction. The remaining 124 markers (49% in female parent, 36% in male parent, and 15% in two parents) were not included because these markers did not accord with the linkage grouping or ordering thresholds (LOD score $\geq 3.0$) of the pseudo-testcross mapping strategy in JoinMap 3.0. Two independent maps were constructed for each *Phalaenopsis* parental cultivar.

The genetic linkage map of *Phalaenopsis* ‘462’ consisted of 122 markers (104 for the male parent, 18 for co-parents) in 15 linkage groups, and had a total genetic distance of 820.28 cM (Fig. 1). The length of the linkage groups varied from 3.67 cM to 168.96 cM, with an average of 6.72 cM between adjacent markers. The largest gap between two markers (37.8 cM) was found on Linkage Group 9. The average number of markers per linkage group was 8.13, ranging from 2 to 57 for each linkage group.

For *Phalaenopsis* ‘20’, a total of 175 (165 for female parent, 10 for co-parents) markers were placed into 14 linkage groups, defining a total genetic distance of 878.29 cM (Fig. 2). The length of the linkage groups varied from 6.2 cM to 181.61 cM. The average distance between adjacent markers was 5.02 cM. The largest gap between two markers was found in Linkage Group 5. The number of markers for each linkage group varied from 2 to 107, with an average of 12.5 markers per linkage group.

**Homologous Linkage Groups**

AFLP loci that were heterozygous in both parents could help bridge homologous linkage groups of the co-parental map. In this study, 23 markers out of 41 shared by both parents were mapped on the parental maps, with 18 markers assigned on the male *Phalaenopsis* ‘462’ map and 10 on the female *Phalaenopsis* ‘20’ map. The homologous group nodes of two parental maps were formed in the five linkage groups. Markers MFP1-M17-450 and MFP13-M38-500 were detected in the linkage groups FLG-1 and MLG-1, markers MFP3-M19-600 in the linkage groups FLG-14 and MLG-1, and markers MFP11-M11-750 and MFP2-M10-500 in the linkage groups FLG-9 and MLG-3 (Fig. 3). Therefore, homologous relationships could exist in the linkage groups of bi-parentals.

**Discussion**

Compared with other markers, AFLP is a dominant PCR-based marker, requiring relatively little template DNA and little prior knowledge of the target template DNA sequence, and reveals high levels of polymorphism in many species.
This molecular marker system has been used in the studies of genetic diversity and gametophyte selection of Phalaenopsis (Hsu et al., 2008; Chang et al., 2009, 2010; Gawenda et al., 2012). The AFLP technique has been widely used for linkage mapping in many plants including sweetpotato (Krieger et al., 2003), velvetbean (Capo-Chichi et al., 2004), apple (Kenis and Keulemans, 2005), wintersweet (Chen and Chen, 2010), crape myrtle (He et al., 2014), and Eucommia ulmoides (Wang et al., 2014). A high level of DNA polymorphism detected by AFLP technique in Phalaenopsis allowed us to construct its genetic linkage map using F1 progenies.

AFLP markers were sensitive to segregation distortion and highly distorted markers had been discovered in many species (Behrend et al., 2013; Chen et al., 2013; Han et al., 2002), which was supposed to be one of the forces for biological evolution (Konishi et al., 1990). The segregation distortion was often much higher in inter-specific populations than in intra-specific populations (Myburg et al., 2003) and caused from biological reasons such as gametes selection, faulty chromosome pairing and so on (Zhang et al., 2010). There were an average 24.42% of the distorted segregation in our study of Phalaenopsis, which was similar to 22.61% in crape myrtle (He et al., 2014), 24.8% in lily (Abe et al., 2002) and 23.5% in rhododendron (Dunemann et al., 1999), and less than 34.8% in wintersweet (Chen and Chen, 2010). Highly distorted segregation markers used to construct genetic maps did not affected marker order and map length (Hackett and Broadfoot, 2003), so some genetic maps were constructed without removing the segregation markers (Ky et al., 2000; Han et al., 2002; Yan et al., 2005; Behrend et al., 2013). Here, maybe the biological cause was the main reason. Most of Phalaenopsis are heterozygous at the gene, including both the parents. Thus, further researches with intra-specific crosses, larger progenies and more molecular markers would be helpful to study the segregation distortion in Phalaenopsis. Considering the addition of distorted markers could increase the identification of regions of interest in further study of Phalaenopsis.

Table 1: The number of polymorphic fragments and mapped fragments generated by each of 71 Psal3/Msel+3 primer combinations in the development of the genetic linkage maps of Phalaenopsis ‘462’ × Phalaenopsis ‘20’

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<th>Number of mapped fragments</th>
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the distorted markers were not discarded and marked with asterisks on the construction of genetic map in this study.

Nineteen link groups were anticipated to be mapped corresponding to the haploid chromosomes number for genus *Phalaenopsis* (2n = 38). However, only 15 linkage groups were obtained in the male map (Fig. 1) and 14 in the female map (Fig. 2) in this study. Similar situation had been reported in other plant species (Mignouna et al., 2002; Chen and Chen, 2010; Xue et al., 2010; Zheng et al., 2013). In this paper, a low number of markers and small population were related to small linkage groups and unlinked markers. With additional markers and larger population, a much more comprehensive genetic map of *Phalaenopsis* would be constructed.

The wide marker space of 20 or even 50 cM may be optimal for QTL mapping scanning (Darvasi et al., 1993). In this study, AFLP markers with the average recombination distance were 6.72 cM and 5.02 cM, respectively for parental linkage maps that provided a favorable situation for QTL scanning.

The clustering of many markers loci to one or two linkage groups intensively seems a common phenomenon in AFLP genetic linkage maps (Ouédraogo et al., 2002; Strommer et al., 2002). This occurred in FLG-1 and MLG-1 of *Phalaenopsis* map. One of the reasons for this intensive clustering phenomenon may be the higher sensitivity of the AFLP technique because the degraded recombination rate near centromere or telomere, non-equal of restriction enzyme sites and high repeat factor could cause these intensive clustering markers (Strommer et al., 2002). AFLP markers from EcoRI/MseI restriction enzymes were found to concentrate often in the vicinity of the centromere of chromosome due to centromeric suppression of recombination (Haanstra et al., 1999; Bonnema et al., 2002), while contrarily the polymorphic markers from PstI/MseI restriction enzymes were distributed more equally than that marker from EcoRI/MseI restriction enzymes in the genome (Pradhan et al., 2003). However, PstI/MseI were used as restriction enzymes in this study. High density clustering of markers still appeared in the linkage groups in FLG-1 and MLG-1. It has been argued that high density clustering may be the results of the recombination or too small mapping population (De la Rosa et al., 2003).
Fig. 3: Homologous linkage groups of both maps are presented side by side. For each linkage group, the names of markers are shown at the left and the marker intervals shown in Kosambi centimorgans (cM) their map position (cM) at the right. For details of AFLP marker nomenclature see Table 1. Asterisks indicate distorted segregation of markers \( (\chi^2 \text{ test}) \)

\[ *p = 0.05, **p = 0.01, ***p = 0.005, ****p = 0.001, *****p = 0.0005 \]

**Conclusion**

A preliminary genetic linkage map was constructed for *Phalaenopsis* by AFLP markers, a species that was relatively lacking the genetic information. To our knowledge, this was the first linkage map for *Phalaenopsis*, which may serve as a tool in QTL analysis, molecular marker assisted selection, and map-based cloning in further study, especially the linkage between molecular markers and valuable genes was the premise for cloning gene such as fragrance, growth habit and flower color, or other single gene traits on the maps. However, this map was not saturated, more additional markers and larger population needed to be used to construct a much more comprehensive genetic map of *Phalaenopsis*.

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**References**


Wang, D., Y. Li, L. Li, Y. Wei and Li Z, 2014. The first genetic linkage map of Eucommia ulmoides. J. Genet., 93: 13–20


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