


QTLs Location of Calyx-related Traits in Tetraploid *Dendrobium*

Ren Yu , Lu Jinghua, Huang Shaohua, Zhang Zhiqun, Kong Xia

Tropical Crops Genetic Resources Institute, Chinese Academy of Tropical Agricultural Sciences, The Engineering Technology Research Center of Tropical Ornamental Plant Germplasm Innovation and Utilization, Hainan Province, Danzhou, 571737, China

 Corresponding author Email: ryhn1016@163.com

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Abstract In order to identify QTL locus of calyx-related traits in *Dendrobium*, 190 F₁ individuals derived from the crossbreed of tetraploid *Dendrobium* cultivars D. Mangosteen x D. Burana Pink No.2 were used as materials. Through three years observation of four characteristics of calyx (length of dorsal sepal, width of dorsal sepal, length of lateral sepal, width of lateral sepal), the QTL was located on the two genetic maps of parents which had been already constructed. The result showed that the four characteristics were quite different among the individuals of F₁ generation, and the observed values of all the characteristics were all positive distribution, which was suitable for QTL analysis. Nine QTLs were detected on the maternal ‘D. Mangosteen’ genetic linkage map, including 1 QTL for dorsal sepal length, 3 QTLs for dorsal sepal width, 2 QTLs for lateral length and 3 QTLs for lateral width, with the genetic contribution rate ranging from 11.9% to 16.8%. Also, two tight linkage markers were acquired (M10E3-146, M8E8-284). A total of 6 QTLs were detected on the paternal ‘D. Burana Pink No.2’ linkage map, including 2 QTLs for dorsal sepal length, 1 QTL for dorsal sepal width, 2 QTLs for lateral length and 1 QTL for lateral width, with the genetic contribution rate ranging from 11.8% to 17.3%. This result of study could provide reference for *Dendrobium* molecular marker assisted breeding and precise location of correlation gene.

Keywords Tetraploid *Dendrobium*; Calyx-related traits; QTL location

Background

Dendrobium is a kind of perennial herb belongs to the genus *Dendrobium* of the Orchidaceae, which is an ornamental with bright market prospect. Among tropical orchid, it is listed as “the top four ornamental foreign orchid” with *Cattleya*, *Phalaenopsis*, and *Vanda* for their high ornamental value (Zeng and Hu, 2004). At present, the breeding of dendrobium is in traditional way. It costs long time and being inefficient. The construction of high-density genetic map and QTL of related traits could improve breeding efficiency, thus shortening breeding time (He et al., 2017). Recent years, the research for *Dendrobium* focused on panting (Sun et al., 2012; Jiang, 2014), tissue culture (Zhao et al., 2012; Jiang, 2014; Wang et al., 2017) and genetic diversity and relationships (Bai et al., 2007; Ren et al., 2013). Because of ploidy’ complexity, which is presented as diploid, triploid, tetraploid and hexaploid, most of the species are diploid ($2n=2x=38$) (Jones et al., 1998), most of the cultivars are tetraploid ($2n=4x=76$) (Liao et al., 2012), so the process of dendrobium genetic map construction research is slow.

At present, the research of *Dendrobium* genetic map construction mainly focused on diploid, Xue et al., (2010) used 90 F₁ individuals of diploid ‘*Dendrobium candidum*’ × ‘*Dendrobium hercoglossum*’ as mapping population, constructing a parental genetic map respectively by using RAPD and SRAP mark. Lu et al., (2012a) used 90 F₁ individuals of diploid ‘*Dendrobium moniliforme*’ × ‘*Dendrobium candidum*’ as construction material, constructing a parental genetic map respectively by using EST-SSR, SRAP, ISSR and RAPD mark. In the same year, Lu et al. (2012b) used 90 F₁ individuals of diploid ‘*Dendrobium candidum*’ × ‘*Dendrobium aduncum*’ as construction material, constructing an integrated genetic map by using EST-SSR and SRAP mark. Feng et al., (2013) used 90 F₁ individuals of diploid ‘*Dendrobium nobile*’ × ‘*Dendrobium moniliforme*’ as construction material, constructing a parental genetic map respectively by using RAPD and ISSR mark. Huang (2007) used 90 F₁ *Dendrobium nobile*

hybrid individuals of *Den. Lucky Gal 'Emito' × Den. Fantasy 'Crown'* as construction material, constructing a *Dendrobium nobile* genetic linkage map by using RADP mark. However, no studies have been reported on QTL location of related traits in *Dendrobium*. By using SRAP, RSAP, SSR, ISSR and tetraploid *Dendrobium* ($2n=4x=76$) as mapping population, the research constructed a parental genetic map respectively. The research aimed to conduct positioning for related traits of calyx on the basis of *Dendrobium Phalaenopsis* complete parental genetic map, which provided references for gene cloning of important traits in *Dendrobium*, accurate positioning and molecular marker assisted breeding.

1 Results and Analysis

1.1 Analysis of main characters of F₁ population

Statistics was conducted for the related traits of 190 individuals' sepal in F₁ population. It is found that there are obvious differences in the length of dorsal sepal, the width of dorsal sepal, the length of lateral sepal and the width of lateral sepal. Continuous variation of statistic was presented as characters of quality and traits. The absolute values of skewness and kurtosis are all less than 1. All traits conform to normal distribution (Figure 1; Table 1).

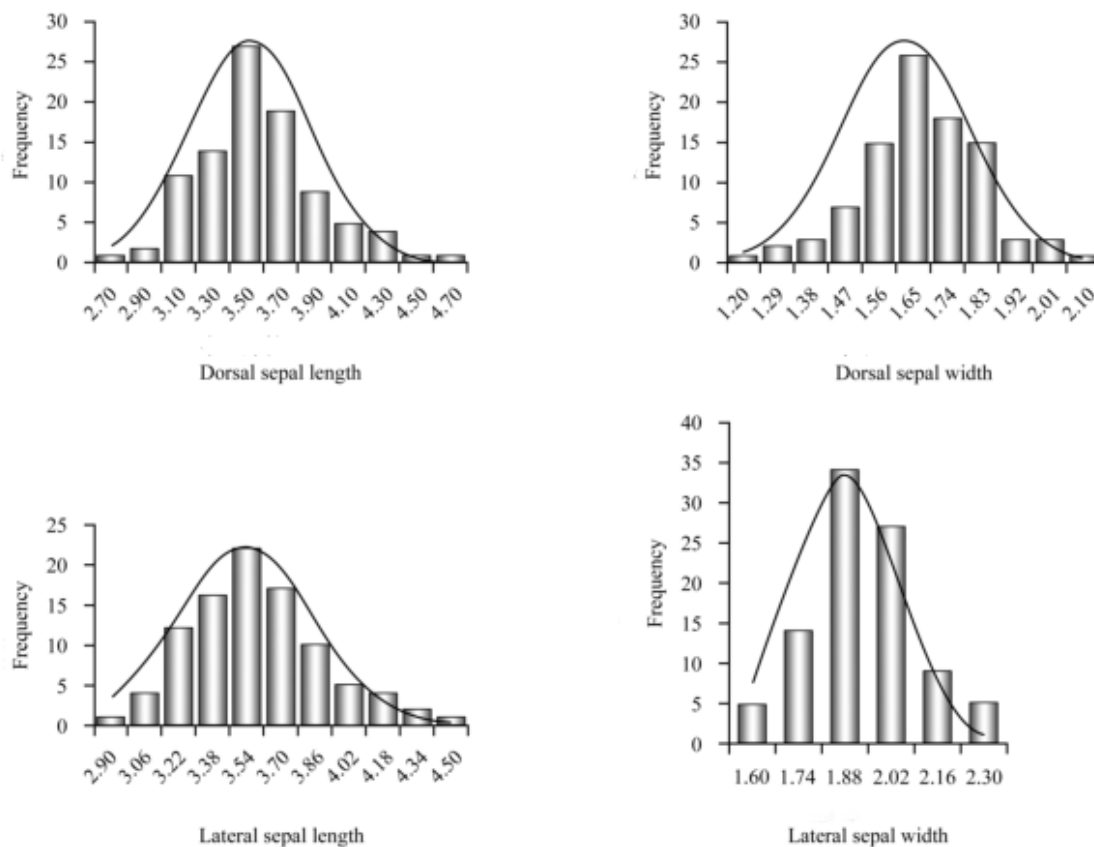


Figure 1 The frequency distribution of 4 traits in F₁ population of *Dendrobium*

Table 1 Parameters of 4 traits in F₁ population

Traits	F ₁ population					
	Max	Min	Mean	S.D.	Kurt	Skew
Dorsal sepal length	4.70	2.70	3.52	0.35	0.95	0.63
Dorsal sepal width	2.10	1.20	1.65	0.17	0.17	0.19
Lateral sepal length	4.50	2.90	3.51	0.32	0.61	0.73
Lateral sepal width	2.30	1.60	1.90	0.17	-0.28	0.35

1.2 QTL analysis of calyx traits

Based on the constructed genetic map, we combined phenotypic data, used MapQTL 6 software to conduct QTL location analysis. Choosing LOD ≥ 2.5 as critical value of QTL, nine QTLs with four traits of dorsal sepal length, dorsal sepal width, lateral sepal length and lateral sepal width were detected in the linkage group of female parents, which distributed in five linkage groups. Six QTL were detected in the male parent linkage group, which were distributed in 2 linkage groups (Table 2; Figure 2; Table 3; Figure 3).

Table 2 The distribution and effect of QTL locus in *D. Mangosteen*

Trait	Trait locus	Linkage group	LOD value	Peak position (cM)	Flanking markers	Distance (cM)	R ² (%)
Dorsal sepal length	Dsl	LGM16	3.75	86.8	R6R10-1121-M10E5-96	3.0	16.8
Dorsal sepal width	Dsw1	LGM10	2.78	32.8	UBC812-600-M6E16-483	2.0	12.7
	Dsw2	LGM13	2.71	27.0	M8E7-509-M3E6-169	2.0	12.4
	Dsw3	LGM21	3.22	41.6	M7E4-148-M13E8-296	7.0	14.6
Lateral sepal length	Lsl1	LGM16	2.75	26.5	M14E4-152-M10E11-249	5.0	12.6
	Lsl2	LGM16	3.28	85.8	R6R10-1121-M10E5-96	2.0	14.8
Lateral sepal width	Lsw1	LGM15	3.26	31.9	M6E4-595-M10E3-146	0	14.8
	Lsw2	LGM16	2.61	60.0	M8E8-288-M8E8-284	0	12.0
	Lsw3	LGM16	2.58	81.5	M9E17-129-R6R10-1121	2.3	11.9

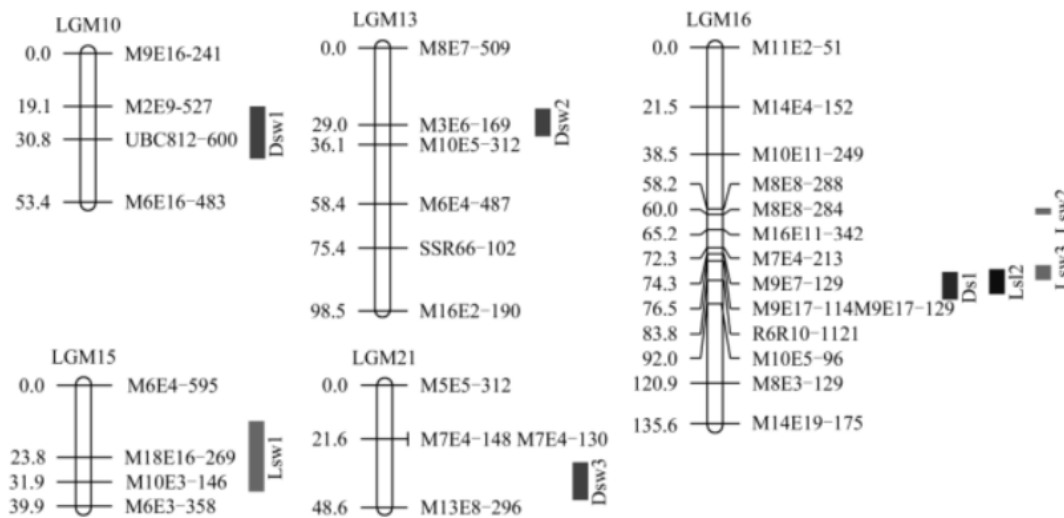


Figure 2 QTL position of female parent *D. Mangosteen* on linkage group

Table 3 The distribution and effect of QTL locus in *D. Burana Pink No.2*

Trait	Trait locus	Linkage group	LOD value	Peak position (cM)	Flanking markers	Distance (cM)	R ² (%)
Dorsal sepal length	Dsl1	LG5	2.72	10.0	M13E8-296-M20E19-15	4.1	12.5
	Dsl2	LG17	3.88	42.0	M16E7-163-M20E16-43	9	17.3
Dorsal sepal width	Dsw	LG5	3.20	4.0	M13E8-296-M8E8-389	4.0	14.5
Lateral sepal length	Lsl1	LG5	2.80	5.0	M13E8-296-M20E19-15	5.0	12.8
	Lsl2	LG17	2.59	39.0	M16E7-163-M20E16-43	9	11.9
Lateral sepal width	Lsw	LG5	2.56	24.1	M20E19-150-M8E8-389	1.7	11.8

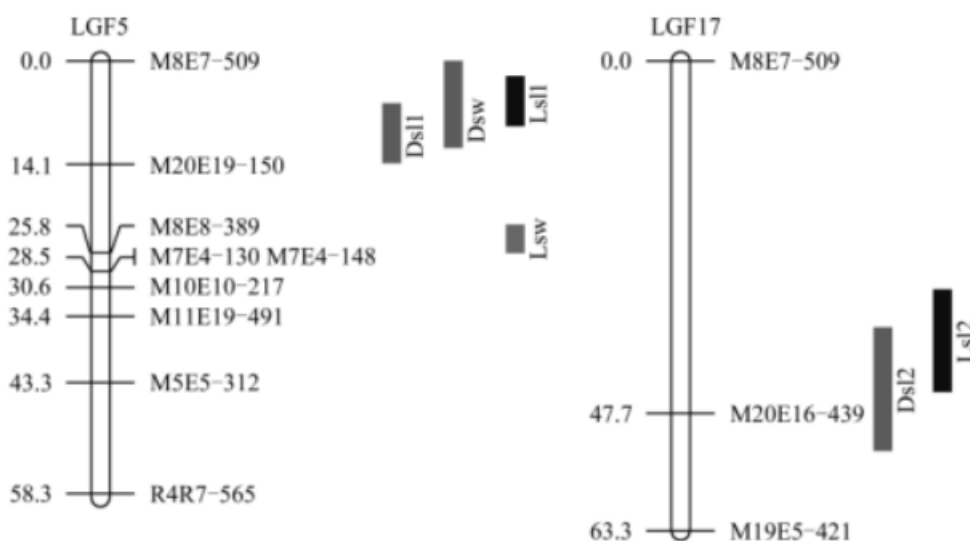


Figure 3 QTL position of male parent *D. burana* Pink No.2 on linkage group

1.2.1 QTL characteristics of maternal traits

Length of dorsal sepal: 1 QTL was detected in linkage group of LGM16, and the location on the linkage group was 86.8 cM, whose genetic contribution rate was 16.8%. The distance between the location and the closest marker was 3 cM (Table 2).

Width of dorsal sepal: 3 QTL were detected in linkage groups of LGM 10, LGM13 and LGM21. The locations on the linkage groups were 32.8 cM, 27 cM and 41.6 cM, whose genetic contribution rates were 12.7%, 12.4% and 14.6%, respectively. The distances between the location and the closest marker were 2.0 cM, 2.0 cM and 7.0 cM (Table 2).

Length of lateral sepal: 2 QTL were detected in linkage group of LGM16, the locations on the linkage group were 26.5 cM and 85.8 cM, whose genetic contribution rates were 12.6% and 14.8%. The distances between the locations and the closest marker were 5.2 cM and 2.0 cM (Table 2).

Width of lateral sepal: 3 QTL were detected, of which one was on LGM15 linkage group and two were on LGM16 linkage group, and the locations on the linkage group were 31.9 cM, 60.0 cM and 81.5 cM, whose genetic contribution rates were 14.8%, 12.0% and 11.9%, respectively. The distance between Lsw1 in LGM15 linkage group and the closest marker M10E3-146 and Lsw2 in LGM16 linkage group and the closest marker M8E8-284 was 0. Which indicated that the two markers and locations were closely linked (Table 2).

1.2.2 QTL characteristics of paternal traits

Length of dorsal sepal: 2 QTL were detected in linkage groups of LGF5 and LGF17, the locations on the linkage group were 10.0 cM and 42.0 cM, whose genetic contribution rates were 12.5% and 17.3%. The distances between the location and the closest marker were 4.1 cM and 5.7 cM (Table 3).

Width of dorsal sepal: 1 QTL was detected in linkage group of LGF5; the location on the linkage group was 4.0 cM, whose genetic contribution rate was 14.5%. The distance between the location and the closest marker was 4.0 cM (Table 3).

Length of lateral sepal: 2 QTL were detected in linkage group of LGF5 and LGF17, the locations on the linkage group were 5.0 cM and 39.0 cM, whose genetic contribution rate were 12.8% and 11.9%. The distances between the location and the closest marker were 5.0 cM and 8.7 cM.

Width of lateral sepal: 1 QTL was detected in linkage group of LGF5, the locations on the linkage group was 24.1

cM, whose genetic contribution rate was 11.8%. The distance between the location and the closest marker was 1.7 cM, which indicated that the marker and the location were closely linked (Table 3).

2 Discussion

Dendrobium is a kind of flower with high ornamental value, and its flower is the most important parts for ornamental and it is the significant goal of its breeding. At present, there is no report on the location of QTL in *Dendrobium* orchids. In the research of tetraploid plants' genetic mapping and QTL location, the potato was mostly reported. Bradshaw et al. (2008) found variation of QTL's number, location and distribution of traits in paternal map in the locating studies of yield, maturity and quality traits of tetraploid potato. Judged from that, the influence of target traits on offspring is also different. Cui et al. (2016) also found similar phenomenon in QTL mapping analysis of three important traits of anthocyanin content, tuber yield per plant and marketable tuber percentage of tetraploid colored potato. It was also suggested that the QTL alleles of the offspring could be analyzed to judge which part of parent had the advantage by the genetic information of the parents. This study had similar results, QTL of 4 characters related to *Dendrobium* calyx was located on the basis of constructing the parents' genetic map by using F₁ group respectively. It was found that 9 QTL locations were detected on the female parent maps of 4 maternal traits, distributing in 5 linkage groups and 6 QTL locations were detected on the male parent map, distributing in 2 linkage groups. The results showed that the number, location and distribution of QTLs detected by these four traits were different in the parent map of *Dendrobium*.

The results also indicated there is a phenomenon called common region between linkage groups of four traits. For example, Lsw3 location of lateral sepal width and Dsl location of dorsal sepal length and Lsl2 location of lateral sepal length detected in the maternal map were in continuous extents; with Dsl location of dorsal sepal length and Lsl2 location of lateral sepal length were located in the same region of R6R10-1121-M10E5-96 on 16 linkage groups (Table 2); However, two locations of dorsal sepal length and two locations of lateral sepal length detected on male parent map were all located in the same segment (Table 3). It is indicated that these two traits might have a close relationship. There was similar phenomenon in potatoes (Schafer et al., 1998; Cui et al., 2016) and peanuts (Chen et al., 2015).

It is also found that QTL clustering is common in different linkage groups. For example, three traits of the dorsal sepal length, the lateral sepal length and the lateral sepal width, appeared in clusters in the 16th linkage group on the maternal map (Figure 2). Four traits of the dorsal sepal length, the dorsal sepal width, the lateral sepal length and the lateral sepal width, clustered on the 5th linkage group on the paternal map (Figure 3). Multi-trait QTL clusters also exist in other crops (Portis et al., 2014; Chen et al., 2015; Ge et al., 2015), it may be the phenotypically related traits were regulated by one or a few polygenic genes and co-segregated genetically. This can effectively increase gene utilization and reduce gene loss caused by recombination (Liu et al., 2013; 2016). Furthermore, the distance between Lsw1, Lsw2 location of lateral sepal width and adjacent markers M10E3-146 and M8E8-284 was 0, these 2 loci could be thought to be closely linked to molecular markers. This provided references for fine mapping and cloning of QTL gene and molecular marker assisted breeding.

3 Materials and Methods

3.1 Materials

190 F₁ individuals of tetraploid D. Mangosteen × D. Burana Pink No.2 were used as experimental material. The material was planted in experiment base of Tropical Crops Genetic Resources Institute, Chinese Academy of Tropical Agricultural Sciences. Each individual was panted in 12 cm×18 cm plastic basin, respectively.

3.2 Observation of agronomic traits

Choosing 190 F₁ individuals between 2014 and 2016, conducting three-year observation and records on the basis of Descriptors and Data Quality Control for *Dendrobium*(*Dendrobium*. Sw) (Yin et al., 2008).

3.3 Data statistics and analysis methods

Statistical analysis was made on the observed values of four characters of the dorsal sepal length, the dorsal sepal width, the lateral sepal length and the lateral sepal width by SAS software. QTL mapping was carried out by using MapQTL 6.0 software on the constructed Genetic linkage map of the parents of tetraploid *Dendrobium*, according to the measurement results of four Calyx traits. Interval mapping was used to analyze genotypes and traits. Software automatically searched for QTL associated with molecular markers when LOD is ≥ 2.5 , drawing QTL map by MapChart 2.2 software (Voorrips, 2006). The nomenclature of the QTL location was represented by “the abbreviation of traits +QTL number”.

Authors' contributions

HSH, ZZQ, KX and LJH were the designers of this experiment and the executors of this experiment and research; LJH was responsible for data analysis, results analysis of the experiment and writing of the first draft of the paper; RY is responsible for the conceiving and he was responsible person of the project, he guided experimental design, data analysis, thesis writing and revision. All authors read and approved the final manuscript.

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