GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

Genetic linkage map of EST-SSR and SRAP markers in the endangered Chinese endemic herb *Dendrobium* (Orchidaceae)

J.J. Lu[1,2]*, S. Wang[1,2]*, H.Y. Zhao[1,2]*, J.J. Liu[1,2] and H.Z. Wang[1,2]

[1]Zhejiang Provincial Key Laboratory for Genetic Improvement and Quality Control of Medicinal Plants, Hangzhou Normal University, Hangzhou, China
[2]Soybean Research Institute, National Center for Soybean Improvement, Nanjing Agricultural University, Nanjing, Jiangsu, China

*These authors contributed equally to this study.
Corresponding author: H.Z. Wang
E-mail: whz62@163.com

Genet. Mol. Res. 11 (4): 4654-4667 (2012)
Received June 8, 2012
Accepted July 24, 2012
Published December 21, 2012
DOI http://dx.doi.org/10.4238/2012.December.21.1

ABSTRACT. *Dendrobium officinale* is an endangered orchid from southeast Asia that is known for its medicinal properties in traditional Chinese medicine. We constructed an integrated genetic linkage map of an F_1 population derived from an interspecific cross between *D. officinale* and *D. aduncum* (both, 2n = 38), using expressed sequence tag-simple sequence repeats (EST-SSR) and sequence-related amplified polymorphism (SRAP). A total of 349 polymorphic loci, including 261 SRAP loci and 88 EST-SSR loci, were identified for genetic linkage analysis. The software JoinMap 4.0 was used to construct the genetic maps. A total of 157 loci were arranged into 27 major linkage groups, each containing a minimum of four markers, and a further 23 markers were distributed to five triplets and four doublets, the frame map covered a total distance of 1580.4 cM, with a mean of 11.89 cM between adjacent markers. This primary map of the *D. officinale* and *D. aduncum* hybrid provides a basis for genetic studies and should

facilitate future studies of medical traits mapping and marker-assisted selection in *Dendrobium* species breeding programs.

Key words: *Dendrobium*; Genetic linkage map; SSR; SRAP

INTRODUCTION

Dendrobium is a monocotyledonous plant in the orchid family, Orchidaceae. There are about 1500 native species all over the world (Tsi, 1980), of which 74 species are mainly distributed in the provinces and autonomous regions of the southern Tsinling Mountains of China (Tsi et al., 1999). Many *Dendrobium* species have been used in traditional medicine for many centuries in Asian countries because of their therapeutic effects (Bulpitt et al., 2007). In traditional Chinese medicine, several *Dendrobium* plants are used as medicines to clear away toxic materials accumulated in human tissues, and as health care products to enhance the immune system. Modern pharmacological studies have shown that *Dendrobium* can promote digestion, slow aging and lower blood pressure and has anti-cancer activity (Chinese Pharmacopoeia Editorial Committee, 2000; Bulpitt et al., 2007), and that it also reduces blood sugar levels (Zheng et al., 2010). As important anti-oxidant elements in *Dendrobium* plants, mucoid substances can moisturize and nourish the skin, and have often been added to beauty products in the modern skin care industry. *Dendrobium* plants not only have a high medicinal value but also a high ornamental value because of its morphology and flowers. However, the ability of self-reproduction in *Dendrobium* is very low (Wang et al., 1999). Because of their wide and effective use in traditional medicine and flower horticulture, *Dendrobium* species are subjected to mass collection, and wild *Dendrobium* resources are being depleted.

Many molecular markers have been used for the development of genetic maps, including restriction fragment length polymorphism, RAPD (random amplification of polymorphic DNA), ISSR (inter-simple sequence repeat), amplified fragment length polymorphism, SSR (simple sequence repeat), and SNP (single nucleotide polymorphism) (Grosse et al., 1999). Among the various molecular markers, SRAP (sequence-related amplified polymorphism) and SSR have proven to be useful for developing and integrating genetic maps for plants. SSR markers are considered to be useful for genetic map construction based on their co-dominance, abundance, and genome-wide distribution. In addition, they generally display good transferability within the genus (Hodgetts et al., 2001; Rajora et al., 2001; Shepherd et al., 2002). SRAP, a new PCR-based marker, has been widely used in map construction, gene tagging, genomic and cDNA fingerprinting, and map-based cloning (Li and Quiros, 2001).

The main objective of the study reported here was to construct the first integrated genetic map of *D. officinale* and *D. aduncum* hybrids based on SSR and SRAP markers by applying the double pseudo-testcross strategy. Our mapping population comprised 140 random samples from interspecies hybrids, thereby representing a perfect experimental population for QTL (quantitative trait locus) and other genetic and genomic studies.

MATERIAL AND METHODS

Plant material and DNA extraction

The female parent *D. officinale* (Sect. *Dendrobium*) was a single wild plant from Ji-

nhua (119°64'E, 29°12'N), Zhejiang, China; the male parent *D. aduncum* (Sect. *Breviflores*) was from a natural region in Luxi (103°76'E, 24°52'N), Yunnan, China. Two flowering plants before hybridization are shown in Figure 1. Both of them were collected from the wild and transplanted in the greenhouse of Hangzhou Normal University (120°19'E, 30°26'N) for further hybridization since 2004. Seeds obtained from cross-pollination were germinated in lightly moistened potting mix at room temperature. Healthy seedlings were transplanted into the nursery garden after three months. Finally, a total of 140 seedlings from interspecies hybrids were randomly selected to construct the molecular genetic linkage map.

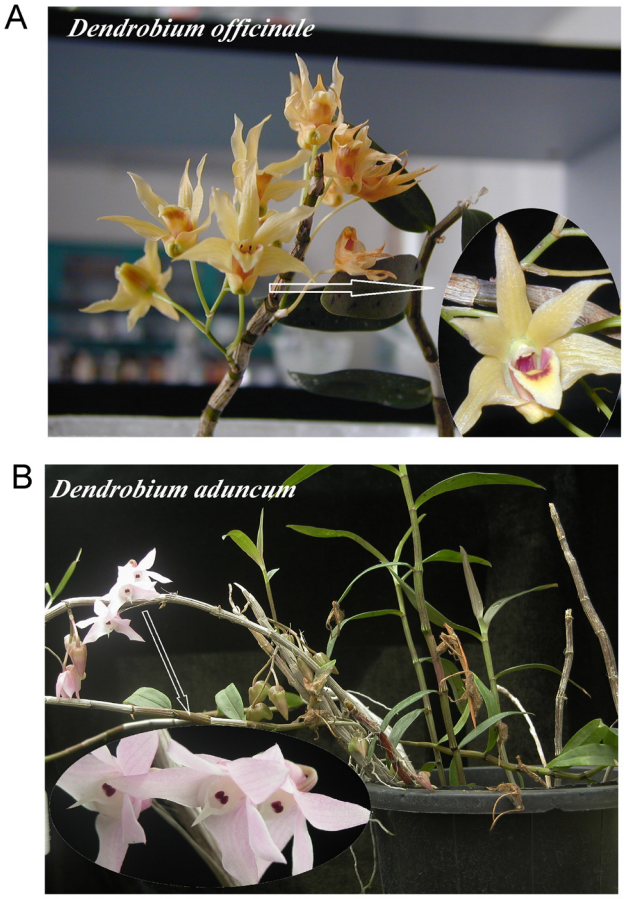

Figure 1. The pictures of maternal (**A.** *Dendrobium officinale*) and paternal (**B.** *D. aduncum*) plants with flower (arrows).

Genomic DNA was extracted individually from leaf samples as described previously (Wang et al., 2009a). The concentration of genomic DNA samples was determined by NanoDrop™ 1000 (Thermo Fisher Scientific, Wilmington, USA) and checked with 1% agarose gel electrophoresis. A portion of the DNA was diluted to 30 ng/μL with solution buffer and used as a template for PCR amplification, and stock portions were stored at -20°C.

Expressed sequence tag (EST)-SSR analysis

A set of 309 pairs of SSR primers were developed based on the sequence data of the ESTs from the cDNA libraries of *D. nobile* (Lu JJ, Kang JY, Ye SR, Zhao HY, et al., unpublished results) and *D. officinale* (Lu et al., 2012b). In brief, the cDNA libraries of *D. noble* and *D. officinale* were constructed, and at least 4075 and 1835 'valid' ESTs were obtained, respectively. The total EST sequences were searched for the presence of SSR motifs by the Perl-based script MISA (http://www.pgrc.ipk-gatersleben.de/misa/), with the parameter for detecting dinucleotide core repeat motifs at least 5 repeats, trinucleotides at least 4 repeats and other SSRs (tetranucleotides up to hexanucleotides) at least 3 repeats. Interrupted composite SSRs were also selected (intervening bases ≤100 bp). The sequences were used to design primers flanking the putative SSRs by Primer 3, with a primer length of 19-22 nucleotides, 40-60% GC content, optimum 50°-60°C melting temperature with a difference of 2°C between forward and reverse primer, and an expected amplification size smaller than 300 bp. The primers were used in amplifying the SSR loci in genomic DNA of *D. noble* and *D. officinale*, respectively, and the sequence and motif of expected size amplified bands for each EST-SSR primer pairs were validated by sequencing. All 309 successfully developed EST-SSR primers were screened with the parental samples to identify the potentially polymorphic loci; the primers showing polymorphism between the parents were then rescreened on six progeny individuals and both parents. Primers with fragments segregating in both parents and the progeny were selected for subsequent use in mapping the whole population.

Amplifications were performed on 96-well microplates using an MJ Research PTC-100 thermal cycler (MJ Research Inc., Waltham, MA, USA). A total volume of 20 µL comprised 30 ng genomic DNA, 0.2 µM each primer, 200 µM dNTPs, 1 U Taq DNA polymerase with 10X PCR universal buffer, and 1.5 mM $MgCl_2$ (TaKaRa, Japan). PCR cycling parameters were 1 cycle of 94°C for 5 min; 32 cycles of 94°C for 40 s, 40 s annealing at temperatures appropriate for each EST-SSR primer pair (Table 1) and 72°C for 90 s, and an extension at 72°C for 10 min. The products were separated on a 6% (w/v) vertical polyacrylamide gel. The gel was run at 220 V constant power for 2 h, until xylene cyanol reached the bottom of the gel. After electrophoresis, the gel was stained with $AgNO_3$ solution.

SRAP analysis

The wonderful transferability SRAP primers, which were developed and used for mapping and gene tagging in *Brassica* by Li and Quiros (2001), were used in our study. Four hundred and thirty-seven SRAP primer combinations were used as candidates, and they were derived from 19 forward primers and 23 reverse primers (Table 2). The high polymorphic SRAP primer combinations used for our map construction were selected with the same selection procedures of EST-SSRs. SRAP PCR mixture was prepared as that in EST-SSR analysis except using different primers, and cycling parameters were: 94°C for 5 min followed by 5 cycles of 94°C for 60 s, 35°C for 60 s, and 72°C for 60 s; 35 cycles of 94°C for 60 s, 50°C for 60 s, 72°C for 60 s, and a final extension at 72°C for 7 min. The SRAP PCR products were separated as for EST-SSR markers as described above.

Table 1. Information of the EST-SSR markers with segregating loci in F_1 population of *Dendrobium officinale* and *D. aduncum*.

Primer name	Primer sequence (5')	Primer sequence (3')	Motif	Tm (°C)	N_A	Linkage group in this study
DNeSSR107	AGGATGTATGGCACTGAGGA	AGGGAGCAGATGATGAACAA	$(GAT)_4$	55	1	
DNeSSR109	TCTATGATGCCGTCTGCTGT	TTTGCTCCAACTCCTCTGTTC	$(CTT)_6$	56	1	D1
DNeSSR114	TCAACTCCCGAAGAAAAGTC	GCACATAGTTCCAACAAATAAAA	$(TA)_7$	55	2	
DNeSSR118	GGGGATGTGCAGTGTTAGAT	GAAGATTGTCCATTCGTTGG	$(AAGAGG)_2$-$(AG)_6$	55	1	
DNeSSR12	GAGAGAGAGAGCGACAGAGA	GATTCTTGGGAGGAAAGATAA	$(GA)_8$	55	2	
DNeSSR123	AAATCATCGCCGTGAGAAGC	CTCCCTGTATTCCAGCATCG	$(AG)_{20}$	59	1	
DNeSSR135	TGGAGTTCGATTGGTGATTA	GGCTACTACTTCGCTTCCTC	$(ATACG)_3$	54	1	
DNeSSR142	CTGATTTGAGGGAAGTGGTA	TGTTCTGTATGTTTGGCATG	$(TCA)_7$	52	2	D7
DNeSSR15	GGTTGAAGTTGTCAATGAAGA	CCAACCATACAAAGAGAATCA	$(CTCGC)_4$	55	2	D19
DNeSSR150	GCCCATGTCTCACGGTTCTC	CAATCCCTCTGTGCTTGCTC	$(GAA)_5$	59	1	
DNeSSR164	CAGGATGCTCTAAGCCAGTA	AAAGAACAATGAAAGGCAAG	$(TC)_8$	53	2	
DNeSSR177	TTCTCCGGGATTCGACTGAT	AGGTTCCTCCTACTTGTGGC	$(CTT)_5$	58	2	
DNeSSR178	TGGATACAGTTCCCATCACCT	AACATACTTCCCAATACCAACG	$(CGG)_5$	57	2	
DNeSSR18	GCCATGAAGTTCCAGATTC	TTGAAATACTTGGACATACGG	$(CCG)_8$	55	1	
DNeSSR180	CAGGGAGGGGAATGAGGTAT	AGAAGAGTAGCACAACGAAAA	$(GTGCT)_4$	55	2	
DNeSSR193	TACGCAATCCTGGTGGAAGA	ACCCACAACCCAGAAACACT	$(GTTT)_4$	58	2	D26
DNeSSR32	GGGAGATAAAGTGAAGGAGAT	CTTTTTGAGGGTGGAGAATAG	$(TTC)_4$-$(TCTT)_4$	55.5	2	
DNeSSR41	TTGTGTAATTGATGTGCATGT	CAACACAAGCTGTGCTTATTA	$(AT)_6$-$(ATGT)_3$	54.5	2	
DNeSSR49	GGCACGTCGAGCCGGAAGAT	GCCCTGGCTTCGAGCTGAGA	$(GA)_8$	66	2	Minor group 6
DNeSSR5	GAGAAGAGGAAGAGCAAGAAT	GCCTAAGAGAAGAAAGTCCAC	$(TCT)_6$	54	2	
DNeSSR50	GAGAAAAGCGAGCGAGCGAG	AGAACCATCGGGCTGGGACT	$(AGCG)_4$-$(GA)_{16}$	63	2	
DNeSSR51	ATCCGCAACTCTACCACCAT	ACATCCTCATCAGCCTTCAT	$(CAG)_5$	55	1	D25
DNeSSR58	ACTACGCGATCAGGTCTGTG	CTCTGGTGCTTACTACAATGCT	$(CTT)_7$	55	2	
DNeSSR59	TGGGCTAACCATTGATTTAC	CAGACTTTATTGTTTGCCTAATT	$(CTTC)_2$-$(TG)_6$	53	2	
DNeSSR6	GGTTTGAAATGTTTCTCATCA	ACTTCTCATTGGTGATCTGC	$(CT)_{13}$	55	1	
DNeSSR75	GAAGAGGGAATCGTGGCAGAA	CGAGGGCAACATACATCAAC	$(TTTG)_3$	58	2	
DNeSSR98	GGGGAGACCGATTTAGGAGA	AGGAGAAGCAACACGAGGAT	$(GA)_7$	57	2	
DOeSSR105	TGGCTTGATGACCACTCTGC	GGGTCGGATTTGAGTCCATA	$(TC)_6$-$(TATC)_4$	58	2	
DOeSSR110	TGGGATTCGCCTGGACAACA	CAGCCAAGATGACCACAAGTAGCA	$(TCT)_5$	64	2	D1
DOeSSR20	CATCCTCCTCCTTCAACTTAT	GCATTCTTCTACTGGTTTCAG	$(GAAGCA)_4$	54	1	D1
DOeSSR28	CGGTGGTCGTACAGAGAG	CTTGTGCTCATGAATCTTCTC	$(GA)_{11}$	55	2	
DOeSSR35	TTCAGTCACATGCTTCTCTCT	ACTGCAAAAATTTACAACAGC	$(TG)_6(TGA)_4$	55	2	
DOeSSR39	TGTCCAGATTCATTTCAGTTT	CTGCAACTTCAAAAATTATGC	$(CTCC)_4(CT)_{12}$	54.5	1	D18
DOeSSR43	CCAAGTCTGAAGTTAATGACG	CCCCCTAACATACCAGAATAC	$(GAA)_4(GAT)_4$	55	1	D9
DOeSSR46	AATGTTGTTGTTCCTTCTGTG	AGCCCAATAGACAAGATAGGT	$(TC)_{14}$	54.5	1	Minor group 7
DOeSSR48	CGCTCTGTTTCTCTCTACCTC	AAGACCTTACGATATTGCACA	$(TC)_8$-$(CT)_7$	55	2	
DOeSSR49	ATGCTCTTCTTCTTCTTTGGT	AAATTCCTCACATTTCAGTCA	$(TC)_{13}$	54	1	D13
DOeSSR51	TAACTAGAAGCCTTTGCTCAG	GAAGCTCCATGTTAATCACAG	$(CT)_{13}$	54	2	
DOeSSR52	CTACTCTACCGGCGTGCAAAT	AGATCCCTGGAGGAGTATTTT	$(TC)_{10}$	57	1	
DOeSSR6	TCCCAATCCTGAAATCTATAA	GTAGGAAGAGACGAGGAGAAG	$(TC)_{11}$	54	2	
DOeSSR61	AATGTCGTAACTTCTGATCCA	GTTCCCCTTGCTCTGTTC	$(CT)_{16}$	55	1	D24

Continued on next page

Table 1. Continued.

Primer name	Primer sequence (5')	Primer sequence (3')	Motif	Tm (°C)	N_A	Linkage group in this study
DOeSSR62	CCTTCTTCTCATTTGTCCTTT	GACCACCATGAGAAAGTCATA	$(TTTGT)_4$	55	2	
DOeSSR65	TAGCTTATCGTGAGAGATTGG	GTCAGGCACATCACAAATAAT	$(AG)_{15}$	55	2	D3
DOeSSR66	GAGGCCTCTCTCCGTTTC	CTGTTTCTTCTTTCTTTGTGC	$(CTTC)_3$-$(AACCCT)_3$	49	1	D7
DOeSSR73	GTTTCAGTCTCTGCTGGTCTA	CATCCAATATTTCTTTGCTTG	$(AG)_{16}$	55	1	
DOeSSR75	GGATTCGATTTGAGTGAATAG	TCACCACAACATTATACATACA	$(AAT)_9$	54	1	D1
DOeSSR77	AGAAACCAGGTCCAATCTCT	ATTTTGTTTCTGGCGAACTAT	$(TC)_{15}$	55	2	
DOeSSR78	TTCAGAGTTCTTGGTGACATT	CAGACCCAAACTCTACCTGTT	$(TC)_6$-$(TCTA)_5$	55	2	D1
DOeSSR79	CTCTACCTCACCGTGTTCCG	CTCTTGCCATTGCTCTGCTT	$(AG)_9$	58	2	D10
DOeSSR8	GAGCTTAACTGCAGAAGATCA	CAGCACAGCATAGCTTATGTA	$(GAAGCA)_6$	54	1	
DOeSSR83	CAGCGACAGCCCAAATCCT	CTTCAGCCTCGTCATCCACC	$(CCG)_3$-$(GGA)_3$	60	1	Minor group 5
DOeSSR85	AGGAGGTGAAGAATGGGAGG	AACAGCAGCATCAATCAAATAG	$(AG)_8$	56	2	
DOeSSR92	AGTTGATAGGACCTTTGTGG	AACTCTGAAACTTTCCCTGA	$(CA)_5$	51	1	D10
DOeSSR93	TGGTTCTATCTTACCTCGGA	GATTGTTTTATTCTGATTTGCA	$(TGT)_7$	53	1	
DOeSSR94	CTGAGATGGACGGCGGTTTC	TCATTGCCCAACGGAAGGAA	$(GA)_5$	63	2	
DOeSSR97	CTTTCTACCACCAGGTTGTCA	CCATAAAGTCGGTCCATAGC	$(CT)_7$	55	1	

Tm = melting temperature; N_A = number of polymorphism loci per primer pair.

Table 2. Sequences of SRAP primers used in development of genetic linkage maps for *Dendrobium*.

Primer name	Sequence of primer (5'→3')	Primer name	Sequence of primer (5'→3')
ME 1	TGAGTCCAAACCGGATA	EM 1	GACTGCGTACGAATTAAT
ME 2	TGAGTCCAAACCGGAGC	EM 2	GACTGCGTACGAATTTGC
ME 3	TGAGTCCAAACCGGAAT	EM 3	GACTGCGTACGAATTGAC
ME 5	TGAGTCCAAACCGGAAG	EM 4	GACTGCGTACGAATTTGA
ME 6	TGAGTCCAAACCGGTAA	EM 5	GACTGCGTACGAATTAAC
ME 7	TGAGTCCAAACCGGTCC	EM 6	GACTGCGTACGAATTGCA
ME 8	TGAGTCCAAACCGGTGC	EM 7	GACTGCGTACGAATTCAA
ME 9	TGAGTCCAAACCGGTAG	EM 8	GACTGCGTACGAATTCTG
ME 10	TGAGTCCAAACCGGTCT	EM 9	GACTGCGTACGAATTCGA
ME 11	TGAGTCCAAACCGGACA	EM 10	GACTGCGTACGAATTCAG
ME 13	TGAGTCCAAACCGGACT	EM 11	GACTGCGTACGAATTCCA
ME 14	TGAGTCCAAACCGGAGG	EM 12	GACTGCGTACGAATTTCA
ME 15	TGAGTCCAAACCGGAAA	EM 13	GACTGCGTACGAATTTAG
ME 16	TGAGTCCAAACCGGAAC	EM 14	GACTGCGTACGAATTCAT
ME 17	TGAGTCCAAACCGGAGA	EM 15	GACTGCGTACGAATTCTA
ME 18	TGAGTCCAAACCGGCAT	EM 16	GACTGCGTACGAATTCTC
ME 19	TGAGTCCAAACCGGAGT	EM 17	GACTGCGTACGAATTCTT
ME 22	TGAGTCCAAACCGGATT	EM 18	GACTGCGTACGAATTGAT
ME 24	TGAGTCCAAACCGGCAC	EM 19	GACTGCGTACGAATTGTC
		EM 20	GACTGCGTACGAATTCCT
		EM 21	GACTGCGTACGAATTAAA
		EM 23	GACTGCGTACGAATTACA
		EM 27	GACTGCGTACGAATTAGA

SRAP = sequence-related amplified polymorphism.

Marker scoring and marker nomenclature

Segregating markers were scored as '1' for presence and '0' for absence of a specific band amplified over the parents and the sibs. The data set included two segregation patterns: 1:1 for markers heterozygous in one parent and homozygous in the other one, and 3:1 for dominant (or 1:2:1 for codominant) markers heterozygous in both parents. SRAP markers were named after the primer serial number and the approximate fragment size. Microsatellite markers were named after the primer name.

Segregation analysis and map construction

For each marker, a χ^2 test was performed to identify alleles that deviated from Mendelian segregation ratios, only the markers with $P > 0.05$ were used for further analysis. Linkage groups (LGs) of the F_1 were established with the JoinMap 4.0 software (Stam, 1993). Markers were placed into LGs with the "LOD grouping" command using the Kosambi map function (Kosambi, 1943). Calculation parameters were set for the minimum LOD threshold of 4.0. The maps were constructed based on the 1:1 segregating markers and 3:1 (or 1:2:1) markers. Distorted markers were not included from linkage analysis. LG drawings were performed with the MapDraw 2.1 software (Liu and Meng, 2003).

Estimating genome length and map coverage

The estimated genome length (Ge) was the sum of the length of each LG multiplied by $(m + 1) / (m - 1)$, in which *m* ($m \geq 4$) is the number of markers in each LG (Chakravarti et al., 1991). The linkage map length (G) was the sum of the length of each LG (more than three markers). Therefore, G/Ge calculates the linkage map coverage.

RESULTS

SSR and SRAP markers

Of the 309 pairs of SSR primers tested in this study, 97 (31.39%) did not show PCR products, 156 of them (50.49%) produced PCR bands without segregation, and the other 56 (18.12%) generated 88 segregating loci, including 24 pairs (44.83%) generating one locus and 32 pairs (55.17%) generating two loci (Table 1). One hundred and three of the 437 SRAP primer combinations displayed better polymorphisms (19 forward primers in combination with 23 reverse primers). The 103 primer combinations generated a total of 261 polymorphic fragments, and the number of segregating fragments per primer combination ranged from 2 to 7, with the size between 100 and 800 bp. Examples of polyacrylamide gel electrophoresis patterns of SSR and SRAP markers are shown in Figure 2.

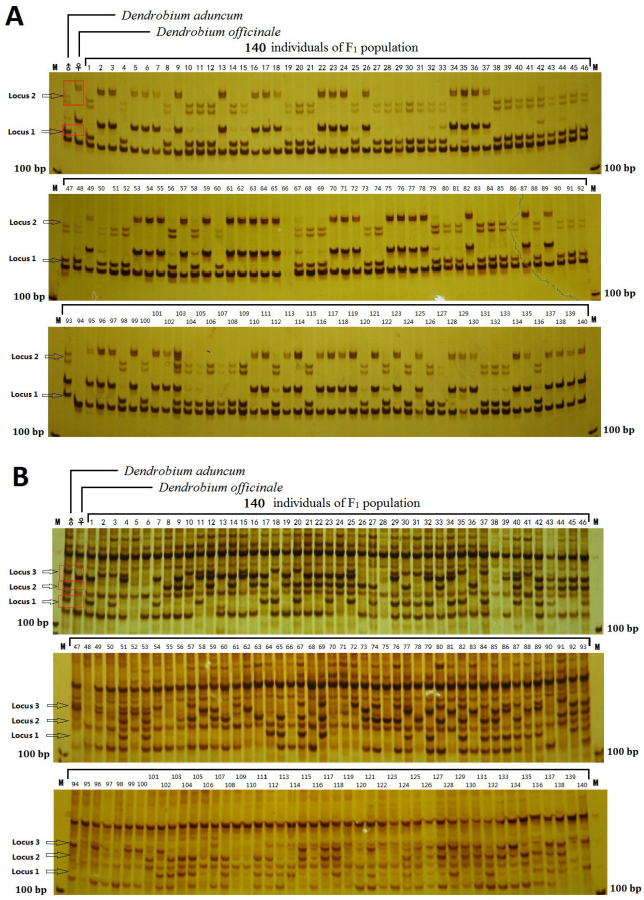

Figure 2. Polyacrylamide gel electrophoresis patterns of SSR and SRAP markers. *Lane M* = size marker; *lane* ♂ = *Dendrobium aduncum*; *lane* ♀ = *D. officinale*; *lanes 1* to *140* = 140 individuals of the F_1 population. **A.** Pattern of *D. officinale* EST-SSR DOeSSR79. **B.** Pattern of SRAP combination M15+E8.

Segregation distortion

In the total number of 349 polymorphic loci (88 derived from SSR primers and 261 derived from SRAP primers), 230 loci showed 1:1 segregation ratio and 35 loci showed 3:1/1:2:1 (14 of them are located on the map and marked with asterisks in Figure 3), and segregation distortion from the expected 1:1 was observed in 63 loci, while segregation distortion from the expected 3:1/1:2:1 was observed in 16 loci, and the other 5 loci showed abnormal separation (Table 3).

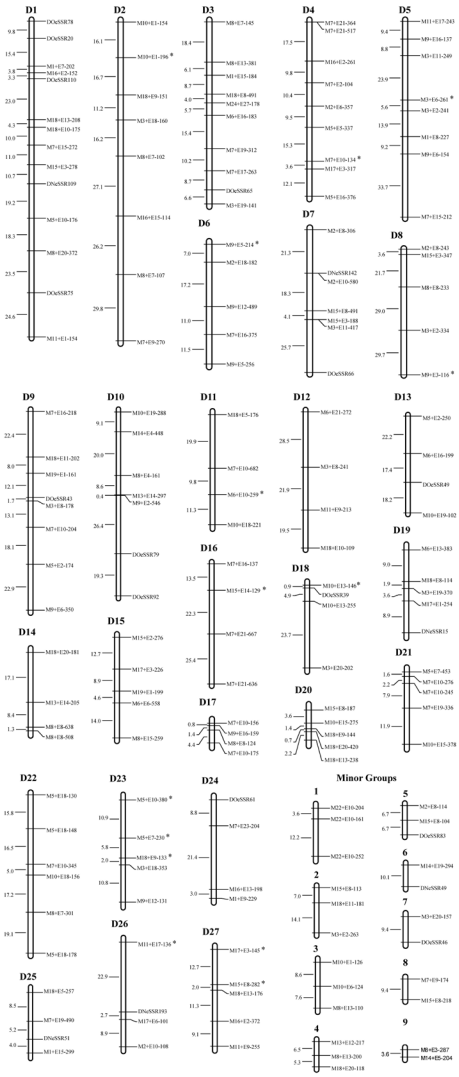

Figure 3. Integrated genetic linkage map of *Dendrobium officinale* and *D. aduncum* hybrid based on EST-SSR and SRAP markers. Numbers at left indicate absolute marker position in Kosambi map units and marker names are to the right of each linkage group. Triplets and doublets are shown as "minor groups". The markers with asterisks mean the 3:1 segregation markers.

Table 3. Number and proportion of marker segregation types during map construction.

Types	Number	Proportion (%)
Total markers	349	
1:1 segregation	230	65.90
Segregation distortion from 1:1	63	18.05
3:1 segregation	35	10.03
Segregation distortion from 3:1	16	4.58
Abnormal separation	5	1.43

Genetic map construction

A total of 265 loci (230 of 1:1 and 35 of 3:1/1:2:1 segregation ratio) were used to calculate the average pairwise LOD and REC data. The final map contained 180 markers (20 SSRs and 160 SRAPs) mapped on 27 LGs with at least 4 markers, 5 triplets and 4 doublets (Figure 3). The map covered a linkage map length of 1580.4 cM with a mean distance of 11.89 cM between adjacent markers (Table 4). The length of the LGs ranged from 6.6 to 176.9 cM, and the number of markers per group varied from 4 to 14. LG D1 contained the largest number of markers (14), whereas LGs D11, D12, D13, D14, D16, D17, D18, D24, D25, and D26 had the least, with only 4 markers each. On average, each LG contained 5.81 markers. The longest gap of 33.7 cM was found in LG D5 (Figure 3).

Table 4. Main characteristics of linkage groups of *Dendrobium* integrated map.

Linkage group	Marker number	Total genetic distance (cM)	Average distance between adjacent marker (cM)
D1	14	176.9	13.61
D2	8	143.3	20.47
D3	10	83.8	9.31
D4	9	78.2	9.78
D5	8	104.5	14.93
D6	5	46.7	11.68
D7	7	69.4	11.57
D8	5	84	21.00
D9	8	98.3	14.04
D10	7	83.8	13.97
D11	4	41.0	13.67
D12	4	69.9	23.30
D13	4	57.8	19.27
D14	4	26.8	8.93
D15	5	40.2	10.05
D16	4	61.2	20.40
D17	4	6.6	2.17
D18	4	29.5	9.83
D19	5	23.4	5.85
D20	5	7.9	1.98
D21	5	23.6	5.90
D22	6	73.6	14.72
D23	5	29.5	7.38
D24	4	33.2	11.07
D25	4	17.7	5.90
D26	4	34.5	11.50
D27	5	35.1	8.78
Total	157	1580.4	
Average	6		11.89

Expected and observed genome length and map coverage

The estimated genome length of *Dendrobium* was 2222.5 cM. The frame map length in this study was 1580.4 cM, which covered 71.1% of the genome (or total length of the linkage group was 1691.2 cM for the genome coverage of 76.1%) (Table 5).

Table 5. Genome length and map coverage of *Dendrobium* in this study.

Item	Total
Genome length estimation (cM)	2222.5
Frame map length (cM)	1580.4
Total linkage group length (cM)	1691.2
Frame map coverage (%)	71.1
Total linkage group coverage (%)	76.1

DISCUSSION

In this study, we selected *D. officinale* and *D. aduncum* as parental materials. They belong to the section *Dendrobium* and *Breviflores*. Between them, there is a large divergence in terms of geographical distribution and phenotype, such as shapes and colors of leaf, flower, and labellum. Both species contain the same number of chromosomes (2n = 38), and their genetic relationship is close based on ISSR molecular analysis (Wang et al. 2009a), suggesting the potential to generate a hybrid between these species. Following a number of trials of crosses between several *Dendrobium* species, we were successful in hybrid production between *D. officinale* and *D. aduncum* for the present study.

The pseudo-testcross is typical genetic strategy to construct linkage maps for an F_1 hybrid family (Grattapaglia and Sederoff, 1994). *Dendrobium* plants have a long growth cycle, complex genetic background, highly heterozygous genetic loci and low self-seed set. These features have caused difficulty in complete genetic map construction of these plant species. Thus far, related genetic information is relatively limited for *Dendrobium* (Wang et al., 1999). In this study, we created an F_1 interspecific hybrid of two *Dendrobium* species, and 140 seedlings of the F_1 family were used for EST-SSR and SRAP genotyping. As codominant markers, EST-SSR loci were used to integrate the two parental maps into one consensus map by the JoinMap software. We constructed the first integrated genetic map for the *D. officinale* and *D. aduncum* hybrid based on SSR and SRAP markers. The frame map length in this study was 1580.4 cM, which covered 71.1% of the genome.

Construction of a genetic linkage map, especially a high-density genetic linkage map, requires not only an appropriate mapping population, but also a large number of molecular markers. Because of the codominant inheritance patterns, abundance, high polymorphism and good stability, SSR markers have been widely used and thought to be ideal markers for constructing genetic linkage maps, especially for integration of the genetic maps from related species (Testolin et al., 2000). In genetic maps reported previously in *Dendrobium* species (Xue et al., 2010; Lu et al., 2012a), both RAPD and SRAP markers were used, but SRAP was preferred, since RAPD markers were considered to have low reproducibility (Jones et al., 1997). The SSR primers from other plants are difficult to apply in *Dendrobium* species, and SSR data are still rarely available for the genus *Dendrobium*. Although we generated primers

for *Dendrobium* EST-SSR, they revealed a low polymorphism between *D. officinale* and *D. aduncum*. Of 309 EST-SSR markers, 56 (18.12%) generated segregating loci that could be used to construct the map, and the rate was lower than that in *Populus* (23.12%), as reported by Wang et al. (2009b). However, the EST-SSR primer pairs used in our study generated more loci per primer pair (1.55) than those in *Populus* (1.08). In the present study, both EST-SSR and SRAP markers were shown to have moderate distribution in LGs without obvious aggregation phenomenon (Figure 3), suggesting that SRAP and SSR markers were feasible and appropriate for genetic map construction in *Dendrobium* species.

As compared with EST-SSR markers, 437 SRAP primer pairs produced 160 loci for the map construction, indicating that SRAP may be more informative in the study of *Dendrobium*. However, SSRs were very useful for the integration of the parental maps (Achere et al., 2004). Clearly, more molecular markers are still needed to join those unlinked markers and small LGs of the primary map we constructed here. It is not surprising that low EST-SSR polymorphism was revealed in the *Dendrobium* plants. The SSR loci mapped in the present study were derived from ESTs, transcripts of functional genes underlying certain physiological processes. Despite the low polymorphism and low abundance, EST-SSR markers are powerful in identifying gene(s) or genetic loci that underlie traits in candidate-based association genetics. Therefore, the EST-based linkage map may be useful for the identification of both genes and QTL that can be further applied to marker-assisted selection of *Dendrobium*, such as the breeding of high levels of medicinal ingredients.

The haploid chromosome number of *D. officinale* and *D. aduncum* is 19 (Cheng et al., 1985), which is smaller than the number of 27 LGs defined by the SSR and SRAP markers. In theory, the number of linkage groups should be consistent with the number of haploid chromosomes. More linkages than theoretically expected were reported in many other plants such as roses (Dugo et al., 2005), olive (la Rosa et al., 2003), lily (Abe et al., 2002), sweet potato (Kriegner et al., 2003; Oliveira et al., 2007), and pecan (Beedanagari et al., 2005). Clearly, many of these described LGs will become fused when additional markers are included. In some cases, an insufficient number of markers may still give a similar number of LGs as the actual chromosome number, but likely without one-to-one correspondence due to missing information on a subset of the chromosome (Wang and Porter, 2004). Eventually, any saturated genetic map should have the number of LGs equal to the haploid number (Yasukochi, 1998; Oliveira et al., 2007). More advanced genotyping technology, such as high-throughput SNP fingerprint at the whole genome or transcriptome scale would generate a large number of DNA markers, necessary for the incorporation of complete genetic linkage maps.

The genetic map of the *Dendrobium* hybrid constructed in the present study brings new insights into genetic architecture and paves an appropriate basis for further comparative mapping among *Dendrobium* species. This preliminary linkage map for *Dendrobium* species that have limited genetic information, constructed using the EST-SSR and SRAP markers, can provide an important framework for future construction of high-density genetic linkage map. However, it is still necessary to construct a moderately or highly saturated genetic linkage map for successive QTL mapping and marker-assisted selections. In the long term, the present study will benefit positional cloning when tight linkages between molecular markers and important genes are established. In addition, confirming the corresponding relationship between LGs and chromosomes for QTL mapping, physical map construction will be needed in the future.

ACKNOWLEDGMENTS

Research supported in part by the National Natural Science Foundation (#31070298, #30870180), the Zhejiang Scientific and Technological Program (#2008C12081), the Hangzhou Scientific and Technological Program (#20080432T06), and the Qiantang Scholar Program.

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