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Construction of a genetic linkage map and identification of QTL associated with growth traits in *Malus sieversii*

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Abstract

110 F_1 individuals of apple were obtained by crossing between a good quality cultivar 'Red Fuji'of *Malus domestica* and an accession 'Hongrou Apple'of *Malus sieversii*. Using Joinmap3.0, a molecular genetic linkage map of *Malus sieversii* was constructed by 175 SSR and 105 SRAP markers which were integrated into 17 linkage groups and spanned 1299.67cM in genome with an average distance of 4.6cM between the markers. By using interval mapping method,17 QTLs for eight growth traits of *Malus sieversii* were detected, including 2 QTLs for tree height on c1 and c16, 2 QTLs for stem height on c7, 2 QTLs for stem thickness on c3, 2 QTLs for new shoot length on c15 and c16, 2 QTLs for new shoot diameter on c2, 3 QTLs for internode number on c1, c2 and c2, 2 QTLs for internode length on c1 and c2, 2 QTLs for lenticel density on c17, respectively. The phenotypic variations explained by each QTL ranged from 10.15% to 41.66%, and their LOD values varied from 2.54 to 4.53, of which five QTLs were major effect genes (LOD≥3.5).

Key words

Growth traits, *Malus sieversii*, Molecular genetic linkage map, QTL analysis

Introduction

Apple (*Malus domestica* Borkh) is one of the important cultivated fruit trees in northern China, with thousand years of cultural history. Due to complex genetic background, highly heterozygous nature, long life cycle and other reasons, the genetic breeding process of apple is still slow. Most of the quality traits of apple were composed of quantitative traits, which were easily influenced by environment factors. The study of genetic basis of apple would certainly improve these traits, and high-quality genetic map is the basis of studying these complex traits from the molecular level.

The construction of genetic map in apple began in early 1990s. Using RAPD method, the first more complete molecular genetic map in apple was constructed with 35 F1 progeny of 'Rome Beauty' × 'White Angel', which contained 360 isoenzymes and DNA polymorphic markers and the marker spacing of each linkage group was from 10 cM to 15cM. Using RFLP, RAPD, Isozyme, AFLP, SCAR and SSR markers, the genetic linkage maps of both parents were constructed with 152 F1 progeny of 'Prima' × 'Fiesta'. So far, several genetic maps of apple have been published. Research on the construction of genetic map in apple started rather late in China and the achievements were less too. Based on the RAPD markers, the first molecular genetic map of apple in China was constructed. Using SRAP markers, another genetic map of apple was constructed with 94 F1 population of 'talamo' ×'Fuji' as materials, which belonged to 18 linkage groups, covering 1127.6 cM of the genome, with an average distance of 11.5cM.

In the previous study, an apple cultivar “Red Fuji” and *Malus sieversii* “hongrou apple” were used as parents, the F1 segregation population was established by distant

hybridization technology. Based on the SSR and SRAP molecular marker technology, a high density molecular genetic map of apple was built. Using the composite interval mapping method, the QTLs of 8 growth-related traits in apple, such as, tree height, stem height, stem thickness, new shoot length, new shoot diameter, internode number, internode length, and lenticel density were mapped, which provided a theoretical basis for the molecular marker-assisted breeding and related basic research in apple.

Materials and Methods

Plant material and growth trait measurement : A cross between *Malus sieversii* 'Hongrou apple' and apple cultivars 'Red Fuji', using 'Hongrou apple' as pollen parent, was carried out in April, 2007. The fruits were picked and stored until November, 2007. After stratification of seeds, the germinated seeds were planted in the greenhouse. In May 2008, 130 seedlings of cross were planted as seedlings in the field and seedlings were grown on their own roots. In the spring of 2011, false hybrid plants were eliminated by SSR and 110 seedlings along with two parents were chosen to construct the genetic map. In October 2008, 10-15 long shoots were collected for the growth trait measurement.

DNA extraction and PCR analysis : Genomic DNA was extracted from the leaf samples according to the CTAB method described by Doule and Doyle (1990). SSR-PCR amplifications and SRAP-PCR amplifications were performed according to the methods described by Zhang Chun-yu (2007).

Map construction and QTLs analysis : SSR and SRAP markers were first screened using the DNAs from two progeny individuals and both parents. Markers showing polymorphism between parents and segregating in the progeny were further used to the genotype of whole mapping population. Informative markers were scored as present (1) or absent (0) according to their parental origin. All markers were utilized to construct the linkage map. Linkage analysis was carried out using JoinMap 3.0 (Van Ooijen & Voorrips, 2001) with a LOD score of 3.0 and a recombination frequency of 0.40 to provide evident linkage. The recombination frequencies were converted to map distances by Kosambi's mapping function (Kosambi, 1994). QTL analyses were carried out using all markers of the genetic linkage maps. The WinQTLcart2.5 software was used to perform interval mapping (IM) in combination with composite interval mapping.

Results and Discussion

Construction of genetic linkage map : All polymorphic markers were used in linkage analysis. Out of 397 polymorphic markers from both SSR and SRAP analysis, 117 (29.47%) were unmapped to any of the linkage map for their significant distortions from typical Mendelian segregation ratios (Table 1). A total of 280 markers, including 175 SSR and 105 SRAP markers, were assigned to 17 linkage groups and the linkage map covered a total of 1299.663 cM of the apple genome (Table 3). The individual linkage groups ranged from 50.4 cM (C15) to 102.756 cM (C2), with an average length of 76.45 cM. The number of markers on 17 groups ranged from 8 (C9) to 43 (C1), with an average number of 15.3. The average interval distance on 17 Cs ranged from 2.1 cM (C4) to 9.5 cM (C16), with an average genetic distance among loci of 4.64 cM. 35 SSR markers showed significantly distorted segregation at P=0.01, accounting for 12.5%.

Trait phenotypic analysis : In order to identify the QTLs affecting growth-related traits, eight traits including tree height, stem height, stem thickness, new shoot length, new shoot diameter, internode number, internode length and lenticel density were measured. The phenotypic variations of these traits are summarized in Table 2. All the eight growth-related traits segregated continuously. Variation coefficient

Table 1 : Genetic distance and distribution of markers in the linkage groups

Linkage groups	Length (cM)	Total of markers	No. of distorted	Average intervals (cM)
C1	98.236	43	7	2.3
C2	102.756	28	5	3.7
C3	74.843	14	1	5.3
C4	63.905	31	5	2.1
C5	77.452	22	4	3.5
C6	135.227	17	4	7.9
C7	63.718	12	1	5.3
C8	70.394	9	2	7.8
C9	70.652	8	0	8.8
C10	73.792	15	1	4.9
C11	72.381	17	3	4.3
C12	71.468	14	2	5.1
C13	60.672	12	0	3
C14	63.078	10	0	6.3
C15	50.4	10	0	5.1
C16	85.095	9	0	9.5
C17	65.594	9	0	7.3
Total	1299.67	280	35	4.64

Table 2 : Phenotypic analysis of some growth traits for apple F1 family

Traits	Mean	Maximum	Minimum	Range	Variance	Standard deviation	Variation coefficient (%)	Kurtosis	Skewness
Tree height	341	478	241	237	2742.9	52.1	15.3	-0.057	0.425
Stem height	94.7	142	35	107	375.8	19.3	20.4	0.556	-0.388
Stem thickness	3.97	6.05	2.03	4.02	0.598	0.77	19.4	-0.095	0.127
new shoot length	89.9	121.5	63.3	58.2	155.4	12.4	13.8	-0.124	0.456
new shoot diameter	0.795	1.02	0.58	0.44	0.009	0.094	11.9	-0.613	0.103
Internode number	49.9	70	36	34	61	7.78	15.6	0.0009	0.632
Internode length	1.83	2.81	1.17	1.64	0.077	0.276	15.1	1.778	0.632
Lenticel density	43.5	63	32	31	35.9	5.96	13.7	0.697	0.758

Table3 : The QTL distribution of 8 growth traits in *Malus sieversii* genetic linkage map

Traits	QTL	Linkage group	The nearest maker	Position (cM)	Distance (cM)	LOD value	Recombi	R^2 %
Tree height	*sg*-1	C1	CH05c06-102f	61.71	0.4	3.1	0.0041	34.62
Tree height	*sg*-2	C16	CH02f06[1]-130p	48.31	1.99	3.6	0.0194	27.58
Stem height	*gg*-1	C7	CH02g09-102m	27.51	1.76	2.64	0.0174	41.21
Stem height	*gg*-2	C7	CH03a08-147m	44.41	1.04	2.56	0.0104	17.48
Stem thickness	*gc*-1	C3	me7em4-294p	39.61	1.97	2.87	0.0193	11.01
Stem thickness	*gc*-2	C3	CH03d08-94m	48.41	1.58	3.11	0.0156	13.6
New shoot length	*Xsszl*-1	C15	MS02a01-130p	30.61	0.54	3.56	0.0055	23.26
New shoot length	*Xsszl*-2	C16	CH03d07-242f	70.71	7.98	2.92	0.0737	21.32
New shoot diameter	*xscd*-1	C2	me8em2-285m	55.71	0.44	2.58	0.0045	34.38
New shoot diameter	*xscd*-2	C2	CH03d02-235f	80.41	4.05	3.05	0.0388	23.14
Internode number	*jjs*-1	C1	CH05c06-135f	65.01	0.91	2.6	0.0199	11.07
Internode number	*jjs*-2	C2	MS14h03-290f	13.21	3.99	3.97	0.0496	22.12
Internode number	*jjs*-3	C2	CH01f07[a]-185f	55.71	1.19	3.06	0.0357	10.15
Internode length	*jjcd*-1	C1	CH04c07-152m	63.01	0.04	4.53	0.0003	20.88
Internode length	*jjcd*-2	C2	me7em5-435f	67.31	0.46	4.11	0.0004	18.99
Lenticel density	*pkmd*-1	C17	CH01f02[1]-238p	17.61	8.55	2.54	0.0787	41.66
Lenticel density	*pkmd*-2	C17	CH04c07-464m	45.11	8.01	2.59	0.0739	31.91

were between 11.9% and 20.4%, Kurtosis and Skewness were less than 2 which suggested that the ten fruit-related traits in the present study were controlled by multiple genes and thus, were suitable for QTL analysis.

Analysis of QTLs for growth-related traits : 2 QTLs named sg-1 and sg-2 were detected, which affected the tree height. Sg-2 was positioned on C16, whose LOD value was more than 3.5 and which might be a major effect gene. Sg-1 was positioned on C1, whose LOD value was 3.1. Two QTLs, whose contribution rates were 0.41% and 1.94% respectively, explained 34.62% and 27.58% of the phenotypic variance, respectively (Table 1).

2 QTLs for stem height, named gg-1 and gg-2 respectively, were detected which were all located on the linkage group C7 and whose LOD values were all less than 3.5. Two QTLs explained 64.21% and 17.48% of the phenotypic variance respectively, whose contribution rates were 1.74% and 1.04% respectively.

2 QTLs for stem thickness, named gc-1 and gc-2, were detected, which were located on the linkage group C3, and whose LOD values were all less than 3.5 too. The contribution rates of 2 QTLs were 1.93% and 1.56%, which explained 11.01% and 13.6% of the phenotypic variance respectively.

2 QTLs for new shoot length, named xsszl-1 and xsszl-2, were detected. Xsszl-1 was located on C15, whose LOD value was more than 3.5, and which might be another major effect gene. The contribution rate of xsszl-1 was 0.55%. Xsszl-2 was located on C16, whose contribution rate was 7.37%. Two QTLs explained 23.26% and 21.32% of the phenotypic variance respectively.

2 QTLs for new shoot diameter, named xscd -1 and xscd -2, were detected, whose LOD values were all less than 3.5, and were all located on C3 too. The contribution rates of 2 QTLs were 0.45% and 3.88%, which explained 34.38% and 23.14% of the phenotypic variance, respectively.

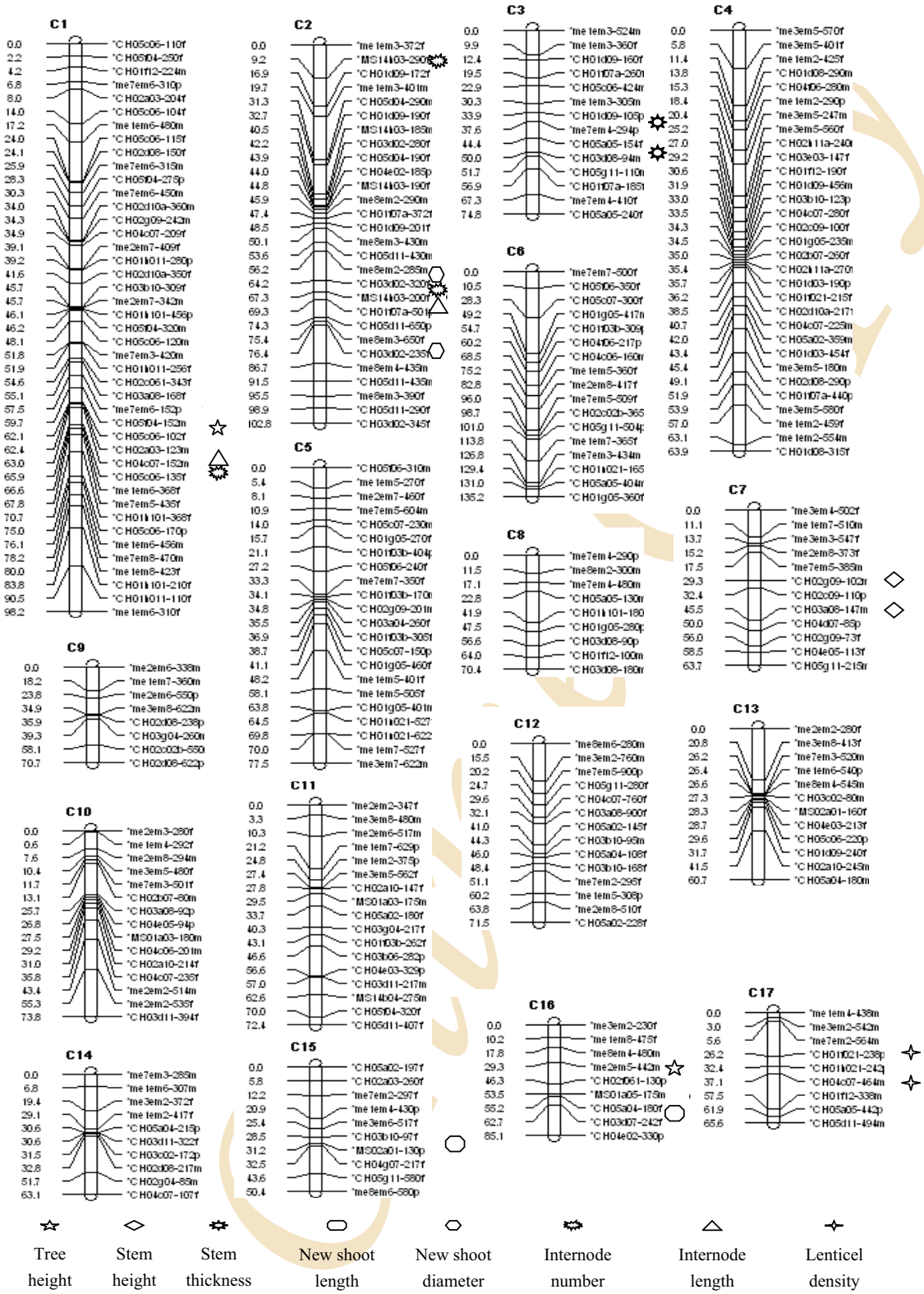

Fig. 1 : QTLs position of growth traits in the genetic map of apple

3 QTLs for internode number, named jjs-1, jjs-2 and jjs-3 were detected. Jjs-2 was located on C2, whose LOD value was more than 3.5, and which might be another major effect gene, which explained 22.12% of the phenotypic variance. Jjs-1 and jjs-3 were located on C1 and C2, which explained 11.07% and 10.15% of the phenotypic variance respectively.

2 QTLs for internode length, named jjcd-1 and jjcd -2, were detected, whose LOD values were all more than 3.5 and which might be a major effect genes. Jjcd-1 and jjcd -2 were located on C1 and C2, whose contribution rates were 0.03% and 0.04% respectively.

2 QTLs for lenticel density, named pkmd-1 and pkmd-2 were detected which were all located on C17. Pkmd-1 and pkmd-2 explained 41.66% and 31.91% of the phenotypic variance, whose contribution rates were 7.87% and 7.39% respectively.

High quality genetic map could define the distance and relationship between genes on chromosomes. Through this genetic map, breeders could select favorable gene to transfer between the species, or transfer new gene from the wildlife resources. With rapid development of molecular techniques, several genetic maps of apple were published. The research on the construction of genetic map in apple was started rather late in China, and the achievements were less. In the present study, using apple cultivar 'Red Fuji' and *Malus sieversii* 'Hongrou apple' as parents, the F1 segregating population was established. A high density genetic map of apple was constructed by the SSR and SRAP molecular marker technology. The genetic map belonged to 17 linkage groups, including 175 SSR and 105 SRAP markers and spanned 1299.67cM in genome with an average distance of 4.6cM between the markers. The length of each linkage group was between 50.4 and 135.2 cM and there were 8-43 markers on the different linkage groups. In comparison to the genetic map of apple, earlier published in China, this genetic map covered more apple genome and further increased the spectrum saturation.

In the present study, 17 QTLs for growth traits were detected, which belonged to 12 different linkage groups and explained the phenotypic variation in 10.15% - 41.66%. The distribution of these QTLs in linkage groups was not uniform, and more QTLs were gathered in the linkage groups C1, C2, C5, C7 and C17 (Fig. 1) and their LOD values varied from 2.54 to 4.53, of which five QTLs were major effect genes (LOD≥3.5). More detailed research on the linkage group segment, in which genes are distributed densely, and further emphasis on the genetic mechanisms would be very significant in future.

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