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Construction of a Genetic Linkage Map and QTL Analysis of Fruit-related Traits in an F1 Red Fuji x Hongrou Apple Hybrid

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Abstract: A genetic linkage map of the apple, composed of 175 SSR and 105 SRAP markers, has been constructed using 110 F1 individuals obtained from a cross between the ‘Red Fuji’ *Malus domestica* and ‘Hongrou’ *Malus sieversii* cultivars, which have relatively high levels of DNA marker polymorphism and differ remarkably in fruit-related traits. The linkage map comprised 17 linkage groups, covering 1299.67 cM with an average marker distance of 4.6 cM between adjacent markers, or approximately 91% of *Malus* genome. Linkage groups were well populated and, although marker density ranged from 2.1 to 9.5 cM, just 10 gaps of more than 15 cM were observed. Moreover, just 12.5% of markers displayed segregation distortion. The present genetic linkage map was used to identify quantitative trait loci (QTLs) affecting fruit-related traits. 23 QTLs for ten fruit traits were detected by multiple interval mapping: 3 QTLs for Vc content, One QTL for single fruit weight, 2 QTLs for peel-phenols content, 2 QTLs for flesh-hardness, 2 QTLs for diameter, 6 QTLs for acid content, 1 QTL for sugar content, 2 QTLs for soluble solids content, 2 QTLs for flesh-phenols and 2 QTLs for brittleness. These QTLs were located on linkage groups C1, C2, C3, C5, C6, C7, C9, C10, C14 and C17, respectively. The phenotypic variations exhibited by each QTL ranged from 2% to 72%, and their LOD values varied from 2.03 to 8.93, of which five QTLs were major effect genes ($R^2 \geq 10\%$). The

tight linkage markers (*me2em7-460f, *MS01a03-180m, *me1em6-307m, *CH05c06-102f, *me1em8-423f) would be helpful to elucidate the molecular mechanisms of apple domestication and breeding in the future.

Keywords: Apple, genetic linkage map, fruit-related traits, QTL analysis

1 Introduction

With a global growing area of almost five million hectares and production of more than 70 million metric tons in 2009, the cultivated apple (*Malus × domestica* Borkh.) can be considered as one of the most widely grown and economically important temperate fruit crops. To maintain this position in the future, new apple cultivars which confer desirable traits such as superior fruit quality and natural disease resistance must be developed continuously. However, genetic studies and breeding for high quality apple cultivars have always been hampered by a long juvenile phase, the space, time and cost involved in maintaining populations, and the strong self-incompatibility present in this species.

Recent advances in DNA molecular markers offer breeders a rapid and precise alternative approach to conventional selection schemes to improve quantitative traits [1]. Using high – quality genetic linkage maps, quantitative trait loci (QTLs) affecting economically important traits could be mapped, genetically evaluated and selected through linked markers, and even cloned and transformed into target plants [2,3]. So far, using PCR-based markers, a number of genetic maps of the apple have been constructed [4-13]. Composed mainly of isozymes, RFLPs, RAPDs, AFLPs, SCARs and SSRs, these have all been developed from scion cultivars or selections. Despite the crucial influence wild *Malus* species have on the fruit quality and disease resistance of cultivated crops, no genetic map has been developed for them until now.

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In recent years, by constructing the molecular genetic linkage maps of the apple, a number of QTLs for many important quantitative traits had been mapped. Using the F1 hybrid populations of ‘Telamon’ × ‘Braeburn’, 74 QTLs of six fruit-important agronomic traits, such as fruit length, fruit diameter, fruit weight, flesh firmness, flesh browning rate and acid content, were mapped [14]. In the same year, QTLs of the growth traits were mapped, and 1.4%-55.3% of the genetic variation was determined to be due to a single QTL (locus) [15]. Using the F1 hybrid populations of ‘Fiesta’ and ‘Discovery’, the QTLs for growth traits and resistance to aphid infestation were mapped in two consecutive years under three environmental conditions [16]. The QTL of fruit firmness were identified, which were distributed among seven different linkage groups [17]. The major QTL for resistance to fire blight was found, which was mapped on the seventh linkage groups [18].

In this paper, we report a new genetic linkage map developed from an F1 cross between the ‘Red Fuji’ cultivar of *Malus domestica* and the ‘Hongrou’ cultivar of *Malus sieversii*, using SSR in combination with SRAP. This map was used to identify QTLs that influence fruit-related traits.

2 Materials and Methods

2.1 Plant material

A cross between the apple cultivars Red Fuji, as the seed parent, and Hongrou, as the pollen parent was carried out in April 2007 (Here, a cross is a cross by distant hybridization technique). The resulting fruits were picked from which the seeds were removed and stored under low temperature sand stratification until November 2007. After stratification, the germinated seeds were planted in a greenhouse. In May 2008, 130 of the F1 seedlings were planted in fields and grown on their own roots. In the spring of 2011, 20 false hybrid plants were identified by SSR and excluded from the study. The remaining 110 true F1 hybrid seedlings, along with two parental trees, were chosen to provide the leaf material for genomic DNA extraction and genetic map construction.

2.2 Harvesting

From September 7 to October 31, 2012, between 10-20 randomly-selected fruits were harvested from each tree, and used for fruit quality measurements. Apples were considered ready to be picked when the seeds had a dark brown color.

2.3 Fruit quality measurements

Fruit quality measurements were carried out on the same day of harvest. The soluble solids content was determined by hand saccharimeter, the flesh firmness was determined with a GY-1 portable hardness tester, the pericarp hardness and brittleness was determined by a TA.XT plus type texture analyzer. The soluble sugar was determined by the anthrone method, titratable acid by the neutralization method, total phenolic content in both peel and flesh by the Folin Ciocalteu colorimetric method, vitamin C content by direct iodimetry. In all cases, reported data represent the mean values obtained of at least ten randomly chosen fruit per plant.

2.4 Data analysis

Statistical analysis was carried out to determine variation, to test distributions by skewness distribution, and to calculate correlation coefficients between individual traits. All statistical analyses were carried out using the commercially available software packages SAS (release 9.1) and Microsoft Excel 2003.

2.5 DNA extraction

Young leaf material (approximately 0.15g) was lyophilised. Genomic DNA was extracted from the leaf samples according to the CTAB method described by Doule & Doyle [19]. The quantity and quality of DNA were evaluated by agarose-gel electrophoresis (1% agarose), and UV transillumination.

2.6 SSR analysis

SSR-PCR amplifications were performed in a 15 µL volume containing 5 ng of genomic DNA, 10 mmol/L Tris-HCl (pH 8.3), 50 mmol/L KCl, 1.5 mmol/L MgCl₂, 200 µmol/L each dNTP, 0.2 µmol/L of each forward and reverse primers, and 1 U Tag polymerase. SSR primers of apple were developed by Swiss Federal Institute of Technology (ETH) and Horticulture Research International (HRI). DNA amplification was performed in a PTC-100TM thermacycler (MJ Research, Watertown, Mass., USA) under the following conditions: an initial denaturation at 94°C for 2 min 30 s followed by four cycles of 94°C for 30 s, 65°C for 1 min, and 72°C for 1 min, and then 30 cycles of 94°C for 30 s, 60°C for 1 min, and 72°C for 1 min. A final cycle of 5 min

at 72°C was included. PCR products were separated by electrophoresis on a 6% denaturing polyacrylamide gel. The gels were silver stained following the protocol of Promega (Promega, Madison, USA).

2.7 SRAP analysis

SRAP-PCR amplifications were performed under the same conditions as SSR-PCR.

2.8 Analysis Segregation analysis and map construction

SSR and SRAP markers were first screened using DNA from two progeny individuals and both parents. Markers showing polymorphism between parents and segregation in the progeny were further used to genotype the 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 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. Markers were assigned to LGs using the 'group' command with a LOD > 4.0 and a max map distance of 20 cM. Linkage groups were assigned as C1-C17, which correspond to the genome linkage groups of apple. The markers were then arranged using 'order' and 'ripple' commands. Linkage map was generated using MapDraw V2.1.

2.9 QTLs analysis

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. A logarithm of odds (LOD) score threshold of 2.5 was used to define QTL significance and an LOD level of >2.5 was set for co-factor selection in subsequent rounds of CIM analysis. QTLs identified in this way were described by the marker with the highest LOD score in the corresponding QTL region. An estimation of the total variance explained by this marker was obtained using WinQTLcart2.5 software, and QTL regions were defined at the 1.0 and 1.5 LOD support intervals, corresponding to a QTL coverage probability of approximately 95%.

3 Results

3.1 Marker polymorphisms

Among 120 tested SSR primer combinations, 64 (53.3%) showed polymorphisms between the parents, and revealed 235 polymorphic fragments whose sizes ranged from 90 to 300 bp. Each SSR primer pair produced two to five polymorphic DNA markers with an average of 3.7 SSR loci per primer pair. Of 235 SSR markers, 220 (93.6%) of them were dominant markers and 15 others were co-dominant markers, accounting for 6.4%. Among all SSR markers, only 41 of them showed a distorted segregation, accounting for 17.4%.

3.2 Construction of genetic linkage map

All polymorphic markers were used in linkage analysis. Out of the 397 polymorphic markers from both SSR and SRAP analysis, 117 (29.47%) were unmapped to any of the Cs because of their significant deviations from typical Mendelian segregation ratios (Table 1). A total of 280 markers, including 175 SSR, and 105 SRAP markers, were assigned to 17 Cs, and the linkage map covered a total of 1299.663 cM of the apple genome (Table 3). The individual Cs ranged from 50.4 cM (C15) to 102.756 cM (C2), with an average length of 76.45 cM. The number of markers on each of the 17 Cs 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%.

3.3 Trait phenotypic analysis

In order to identify the QTLs affecting fruit-related traits, ten traits including Vc content, fruit weight, total phenol peel, flesh firmness, fruit diameter, titratable acid, soluble sugar, soluble solids, total phenols pulp, and peel crispness, were measured in the 110 F1 seedlings and the two parents. The phenotypic variations of these traits are presented in Table 4. The two parents differed markedly in these traits. Transgressive segregations were observed in weight, acid, and SSC falling beyond Hongrou apple, in Vc, peel-phenols, sugar, and flesh-phenols exceeding over red Fuji, in flesh-hardness, diameter, and brittleness beyond both parents. All the ten fruit-related traits segregated

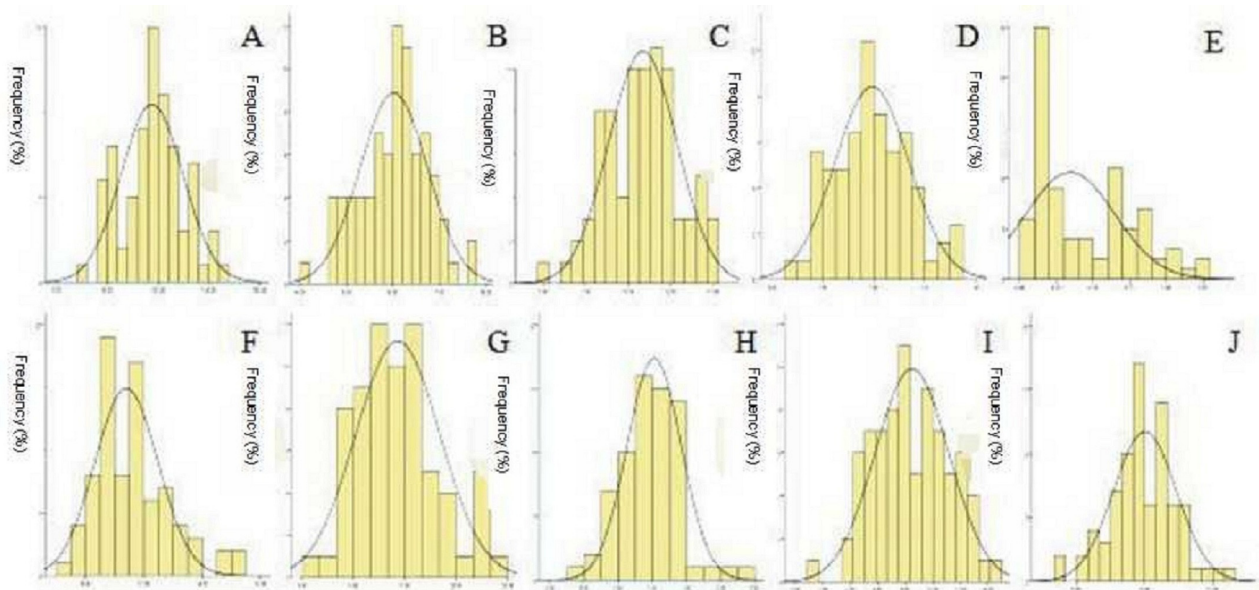


Figure 1. Distribution of number of the ten fruit-related traits in F1. A) Fruit weight histogram, B) Fruit diameter histogram, C) Flesh-hardness histogram, D) brittleness histogram, E) Acid histogram, F) SSC histogram, G) Peel-phenols histogram, H) Flesh-phenols histogram, I) Vc histogram, J) Sugar histogram

Table 1. Primer sequences of SRAP applied in genetic map of *Malus sieversii*.

Code	Forward primers sequence (5'-3')	Code	Reverse primers sequence (5'-3')
me1	TGAGTCCAAACCGG ATA	em2	GACTGCGTACGAATT TGC
me2	TGAGTCCAAACCGG AGC	em3	GACTGCGTACGAATT GAC
me3	TGAGTCCAAACCGG AAT	em4	GACTGCGTACGAATT TGA
me7	TGAGTCCAAACCGG TCC	em5	GACTGCGTACGAATT AAC
me8	TGAGTCCAAACCGG TGC	em6	GACTGCGTACGAATT GCA
		em7	GACTGCGTACGAATT CAA
		em8	GACTGCGTACGAATT CTC

Table 2. The screening of different type of markers used in this study.

Type of markers	Number of screened primer pairs	Number of polymorphic primer pairs	Total number of markers	Number of distorted markers	Number of mapped markers
SSR	120	64	235	41	175
SRAP	100	35	162	30	105
Total	220	99	397	71	280

continuously, and suggested that the ten fruit-related traits in the present study were controlled by multiple genes, and thus suitable for QTL analysis. In addition, the skewness of acid was greater, and frequency chart was significantly deviated from the normal distribution, which was preliminary concluded that the non-equivalent gene or major gene might exist in this trait.

The correlation between fruit traits of F1 population was shown in Table 5. It could be seen from the table, there was different degrees of correlation between

different fruit traits. there was very significantly positive correlation between fruit weight and fruit diameter, between total phenol peel and flesh of total phenols, between flesh firmness and crispness peel, between soluble sugar and soluble solids. There was significant ($P < 0.01$) negative correlation between fruit weight, fruit diameter and flesh firmness. The QTLs of these traits, between which there was significant correlation, might be positioned in the adjacent or close position of the same linkage group.

Table 3. The description of markers and the genetic distance of linkage groups on the map.

Linkage groups	Length of linkage group (cM)	Total of markers	No. of distorted	No. of intervals	Minimum intervals (cM)	Maximum intervals (cM)	Average intervals (cM)
C1	98.236	43	7	42	0.1	7.8	2.3
C2	102.756	28	5	27	0	15.1	3.7
C3	74.843	14	1	13	0.2	10.4	5.3
C4	63.905	31	5	30	0	9.7	2.1
C5	77.452	22	4	21	0	9.8	3.5
C6	135.227	17	4	16	1.6	17.9	7.9
C7	63.718	12	1	11	0	13.1	5.3
C8	70.394	9	2	8	5.6	19.1	7.8
C9	70.652	8	0	7	0.9	18.8	8.8
C10	73.792	15	1	14	0.6	18.5	4.9
C11	72.381	17	3	16	0	11.6	4.3
C12	71.468	14	2	13	1.6	15.5	5.1
C13	60.672	12	0	11	0	20.8	3
C14	63.078	10	0	9	0	18.9	6.3
C15	50.4	10	0	9	1.4	11.1	5.1
C16	85.095	9	0	8	1.7	22.4	9.5
C17	65.594	9	0	8	2.5	20.6	7.3
total	1299.663	280	35	263			4.64

Table 4. Phenotypic variation of the ten fruit-related traits in F1 population and the two parents.

Traits	parents		Variation in the F1 population					
	Red Fuji	Hongrou apple	Range	Mean	SD	CV/%	Kurtosis	Skewness
Vc (mg/100g)	15.45	5.53	5.62-16.31	8.39	2.76	32.9	-0.521	0.07
weight (g)	231.33	97.2	26.1-166.55	94.37	30.73	32.55	-0.418	-0.021
peel-phenols (mg/g)	13.26	7.8	8.33-15.33	13.29	4.1	28.69	-0.151	0.471
flesh-hardness (105 Pa)	6.48	6.75	6.5-6.72	6.54	2.13	19.91	-0.226	-0.128
diameter (mm)	60.99	78.97	41.5-76.45	60.11	7.4	12.13	-0.264	-0.257
acid (%)	0.91	0.28	0.35-0.51	0.44	0.33	75	-0.719	0.652
sugar (%)	8.67	7.74	7.85-8.91	8.58	3.25	23.89	-0.672	0.594
SSC (%)	15.55	11.63	12.9-15.2	13.62	1.29	11.11	0.609	0.764
flesh-phenols (mg/g)	2.03	0.88	0.95-2.1	1.48	0.44	30	0.738	0.236
brittleness (kg/s)	0.73	1.2	0.75-0.92	0.82	0.17	15.05	-0.227	0.489

Table 5. Correlation coefficients among fruit-related traits in the F1 population.

	Vc	weight	peel-phenols	flesh-hardness	diameter	acid	sugar	SSC	flesh-phenols
weight	0.229*								
peel-phenols	0.045	-0.101							
flesh-hardness	0.014	-0.392*	0.312*						
diameter	0.240*	0.862**	-0.052	-0.445**					
acid	0.288*	0.229	-0.026	-0.145	0.199				
sugar	0.413**	0.192	0.248*	-0.092	0.22	0.043			
SSC	0.357*	0.250*	0.320*	0.035	0.208*	-0.077	0.519**		
flesh-phenols	0.066	-0.035	0.613**	0.044	-0.001	0.257*	0.095	0.116	
brittleness	0.0013	-0.248	-0.001	0.492**	-0.353**	-0.172	0.014	-0.165	-0.123

Note: “*” and “**” indicate significances with a probability level of 0.05 and 0.01 respectively.

3.4 Analysis of QTLs for fruit-related traits

Mapping analysis produced 23 putative QTLs which controlling apple fruit-associated traits were located, and a single QTL could explain 2-72% of phenotypic variations (Table 6, Fig 2). These QTLs were distributed on 10 linkage groups, such as C1, C2, C3, C5, C6, C7, C9, C10, C14 and C17, except for linkage groups C4, C8, C11, C12, C13, C15 and C16. The additive effects of 9 QTLs were positive with relation to the Hongrou apple, increasing the effects of QTLs, and the remaining 14 were negative with relation to the Red Fuji apple, increasing the effects. Over half of the collocation QTLs occurred for different traits on linkage groups C1, C5, C6, C7 and C10 (Fig 2). 5 main effective QTL were detected, whose phenotype contribution rate was more than 10%, accounting for 21.8%.

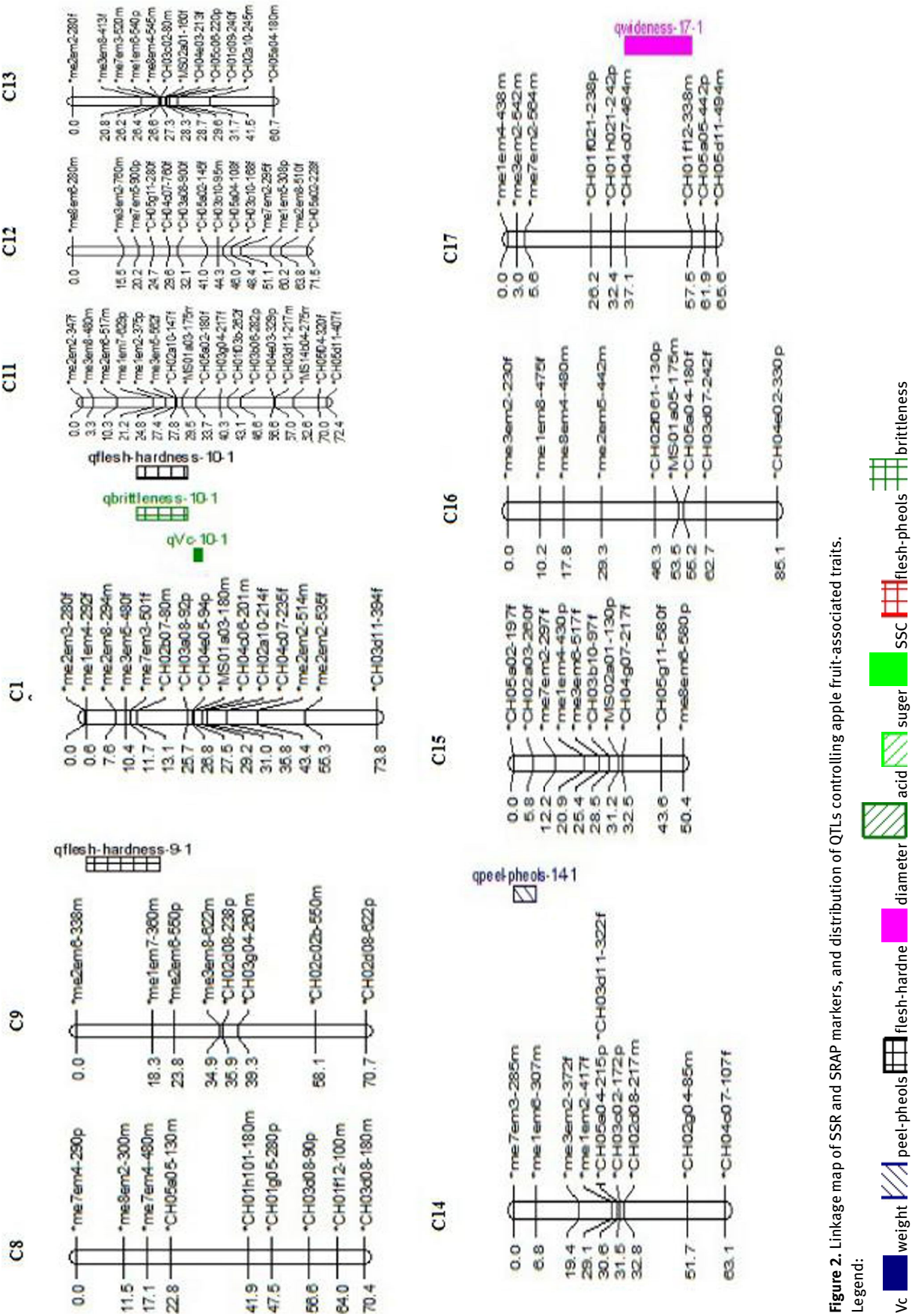
3 QTLs, identified as qVc-5-1, qVc-5-2, qVc-10-1, were detected which affected the Vc content of fruit. Two of the 3 QTLs were positioned on C5, 1 QTL positioned on C10, and the LOD value was in the range of 2.13 to 3.15. qVc-5-1 had a synergistic effect allele from the male Hongrou apple, and the contribution rate was 4.2%, qVc-10-1 had synergistic effect allele from the female red Fuji, and the contribution rate was 5%.

Only 1 QTL for the single fruit weight was detected, which was located on linkage group C6, and identified as qweight-6-1. The LOD value was 2.66. Qweight-6-1 had a synergistic effect allele from the male Hongrou apple, and the contribution rate was 72%, which was the main effect QTL for the single fruit weight.

2 QTLs for peel-phenols content were detected, which were located on linkage groups C5 and C14, identified as

Table 6. QTL analysis for fruit-related traits in F1 population.

Traits	QTL	Linkage group	(cM) Position	The nearest marker	Distance to the nearest marker (cM)	LOD value	Additive	R ²
Vc	qVc-5-1	C5	8.1	*me2em7-460f	0.02	2.13	-0.641	0.042
	qVc-5-2	C5	39.8	*CH05c07-150p	1.06	2.27	-1.044	0.026
	qVc-10-1	C10	27.5	*MS01a03-180m	0.018	3.15	-0.613	0.05
weight	qweight-6-1	C6	77.3	*me1em5-360f	2.05	2.66	-11.03	0.72
peel-phenols	qpeel-pheols-5-1	C5	57.3	*me1em5-505f	0.76	4.38	0.47	0.096
	qpeel-pheols-14-1	C14	6.8	*me1em6-307m	0.019	2.24	4.31	0.465
flesh-hardness	qflesh-hardness-9-1	C9	16.00	*me1em7-360m	2.21	5.25	-0.91	0.095
	qflesh-hardness-10-1	C10	24.10	*CH03a08-92p	1.63	2.68	1.01	0.079
diameter	qdianeter-6-1	C6	75.3	*me1em5-360f	0.052	4.22	-4.13	0.04
	qdianeter-17-1	C17	45.10	*CH04c07-464m	8.00	2.12	2.68	0.039
acid	qacid-1-1	C1	56.2	*CH03a08-168f	1.05	6.28	-0.192	0.034
	qacid-1-2	C1	62.1	*CH05c06-102f	0.007	7.90	-0.195	0.077
	qacid-1-3	C1	74.7	*CH05c06-170p	0.35	2.85	-0.032	0.107
	qacid-1-4	C1	80.0	*me1em8-423f	0.036	2.45	-0.245	0.02
	qacid-6-1	C6	82.3	*me2em8-417f	0.52	2.48	0.047	0.026
	qacid-7-1	C7	21.5	*me7em5-385m	4.04	2.48	-0.116	0.045
sugar	qsuger-2-1	C2	53.7	*CH05d11-430m	0.05	2.12	0.793	0.03
SSC	qSSC-1-1	C1	83.8	*me1em8-423f	0.02	2.95	-0.46	0.033
	qSSC-7-1	C7	14.7	*me2em8-373f	0.52	2.81	0.071	0.06
flesh-pheols	q flesh -pheols-5-1	C5	43.1	*me1em5-505f	0.76	2.35	-0.30	0.104
	q flesh -pheols-5-2	C5	58.1	*me1em6-307m	0.019	4.80	0.22	0.579
brittleness	qbrittleness-3-1	C3	10.00	*me1em3-360f	0.05	2.84	-0.053	0.043
	qbrittleness-10-1	C10	22.10	*CH03a08-92p	3.63	3.52	0.071	0.06



qpeel-pheols-5-1 and qpeel-pheols-14-1 respectively, whose LOD values were 4.38 and 2.24. Qpeel-pheols-5-1 had a synergistic effect allele from the male Hongrou apple, and the contribution rate was 9.6%. Qpeel-pheols-14-1 had a synergistic effect allele from female red Fuji, and the contribution rate was 46.5%, which was the main effect QTL for the peel-phenols content.

2 QTLs for flesh-hardness were detected, which were located on the linkage group C9 and C10, identified as qflesh-hardness-9-1 and qflesh-hardness-10-1, whose LOD values were 5.25 and 2.68 respectively. The contribution rate was 9.5% and 7.9% respectively. The qflesh-hardness-9-1 had synergistic effect allele from the female parent red Fuji.

2 QTLs for diameter were detected, which were located on the linkage group C6 and C17, identified as qdiameter-6-1 and qdiameter-17-1, whose LOD values were 4.22 and 2.12 respectively. Qdiameter-6-1 had synergistic effect allele from the male Hongrou apple, and the contribution rate was 4%. Qdiameter-17-1 had synergistic effect allele from the female red Fuji, and the contribution rate was 3.9%.

6 QTLs for acid were detected, which was identified as qacid-1-1, qacid-1-2, qacid-1-3, qacid-1-4, qacid-6-1 and qacid-7-1. The LOD value was in the range of 2.45 to 7.9. Qacid-1-1, qacid-1-2, qacid-1-3, and qacid-1-4 were all located on the linkage group C1. Qacid-6-1 was located on the linkage group C6, and qacid-7-1 was located on the linkage group C7. Qacid-1-2 and qacid-6-1 had synergistic effect allele from the male Hongrou apple, and the contribution rates were 7.7% and 2.6% respectively. Qacid-7-1 had synergistic effect allele from the female red Fuji, and the contribution rate was 4.5%.

1 QTL for sugar was detected, which was located on the linkage group C2, identified as qsuger-2-1, and whose LOD value was 2.12. Qsuger-2-1 had synergistic effect allele from the female red Fuji, and the contribution rate was 3%.

2 QTLs for soluble solids content were detected, which were identified as qSSC-1-1 and qSSC-7-1. QSSC-1-1 was located on the linkage group C1, and the LOD value was 2.95. QSSC-7-1 was located on the linkage group C7, and the LOD value was 2.81. QSSC-1-1 and qSSC-7-1 all had synergistic effect allele from the male Hongrou apple, and the contribution rate was 3.3% and 6% respectively.

2 QTLs for flesh-phenols were detected, which were all located on the linkage group C5, and identified as qflesh-pheols-5-1 and qflesh-pheols-5-2 respectively. Qflesh-pheols-5-2 had synergistic effect allele from the female red Fuji, and the contribution rate was 57.9%, which was the main effect QTL for the flesh-phenols.

2 QTLs for brittleness were detected, which were

identified as qbrittleness-3-1 and qbrittleness-10-1 respectively. Qbrittleness-3-1 was located on the linkage group C3, whose LOD value was 2.84, and the contribution rate was 4.3%. Qbrittleness-10-1 was located on the linkage group C10, whose LOD value was 3.52, and the contribution rate was 7.1%.

4 Discussion

High quality genetic maps define the distance and relationship between genes on linkage groups. Through high quality genetic maps, breeders are able to select favorable genes to transfer between species, or transfer new genes from wild varieties. The construction of genetic maps in fruit started 1994 [4]. With the rapid development of molecular biology techniques, great progress has been made in recent years, resulting in genetic maps being constructed for numerous fruit trees, including peach [20,21], pear [22,23], citrus [24], and others [25-27]. These days, genetic maps for F1 hybrids of apple are being constructed using various cultivated varieties for both parents, as the closer genetic relationship could highlight more discrete differences. Because the spectra density of the maps was relatively unsaturated, and the marker species were lacking, in-depth differences were difficult to identify [28,29].

In this current study, the apple cultivars 'Red Fuji' and 'Hongrou' were used as parents, with the F1 segregating population established by distant hybridization technique. A high density genetic map was constructed using SSR and SRAP molecular marker technology. The genetic map was made for 17 linkage groups, comprising 175 SSR markers and 105 SRAP markers, with a genome spanning 1299.67cM and an average distance of 4.6cM between markers. The length of each linkage group was between 50.4 ~ 135.2 cM, and there were 8 ~ 43 markers on different linkage groups. Relative to previously published genetic maps published in China, the one presented in this study includes a wider extent of the genome which further increases the spectrum saturation.

Due to chromosome rearrangement, deletion, and other factors in genetic replication, a large number of markers often appeared to be segregation in the process of constructing the genetic map [27,30]. In this study, 71 markers were observed as segregation products, and 35 segregated markers were positioned to the map, accounting for 12.5% of the total number of markers in the map. In all segregating markers, 12 markers (34.2%) were more closely aligned with the male "Hongrou" parent, and 23 markers (65.7%) with the "Red Fuji" parent, indicating

that the segregation in the female was more frequent than in the male. From the distribution of the segregation markers, it could be seen that 35 distorted markers were mainly distributed in 11 linkage groups, and most of the distorted markers showed different degrees of aggregation phenomenon in the linkage groups. This phenomenon was also found in other crops, such as longan, and peach [22,27].

23 QTLs for fruit traits were detected, which belonged to 10 different linkage groups, and explained phenotypic variation in 2% - 72%. The distribution of these QTLs in linkage groups was not uniform, and the QTLs gathered primarily in linkage groups C1, C5, C6, C7 and C10. 2 QTLs for titratable acidity and soluble solids clustered in the linkage group C1, and gathered at the region of 80-83.8 cM. 4 QTLs for Vc content, total phenolic content and flesh peel total phenol content clustered in the linkage group C5, and gathered at the region of 39.8-43.1 cM and of 57.3-58.1 cM respectively. 2 QTLs for fruit weight and fruit diameter clustered in the linkage group C6, and gathered at the region of 75.3-77.3 cM. 2 QTLs for peel and flesh firmness crispness clustered in the linkage group C10, and gathered at the region of 22.1-24.1 cM. The phenomenon of several QTLs controlling different traits positioned at the same location correspond to previously published data [22]. The most likely cause for this phenomenon is gene pleiotropism, the influence of one gene on multiple phenotypic traits, variously activated as part of certain linkage actions or groups [31,32]. QTLs controlling certain traits were mostly clustered at similar or identical regions in the same linkage groups. In this study, 4 QTLs for titratable acid content clustered in the linkage group C4, but the spacing between each other was more than 20cM, indicating it might not be a gene. However, the gene cluster was related to acid metabolism, which was also distributed in this region. Results suggest that this section of the genome may include one or several genes associated with fruit traits. More detailed research on the linkage group segment, in which genes were distributed densely, and discussing the genetic mechanisms are necessary.

Based on the offspring of “Wijcik” and “Golden Delicious”, 17 linkage groups were constructed to map quantitative trait loci (QTL). 1 QTL for fruit weight was detected in the sixth linkage group, and 1 QTL for fruit texture was detected in the tenth linkage group. Another QTL associated with fruit texture was observed in the tenth linkage group too. In this study, the related trait loci were also detected on these linkage groups, but the distance was quite different from Katrien’s. In addition, the linkage group, on which some related-trait

loci were detected, was even inconsistent. The reasons for the difference were different populations, different markers and environmental impacts. The linkage groups corresponding with the number of chromosomes also had a certain relationship.

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