

QTL Analysis and Development of HRM Markers Associated with Fruit Shape in Interspecific Pears (*Pyrus pyrifolia* × *P. bretschneideri*)

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Research Article

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Abstract

Fruit shape is one of the important quantitative traits in pear (*Pyrus* spp.) breeding program, thus genetic study related to fruit shape could be beneficial to pear breeding. Quantitative trait loci (QTL) analysis was carried out using 'Whangkeumbae' (*P. pyrifolia*, round) × 'Yali' (*P. bretschneideri*, pyriform) and high-resolution melting (HRM) markers were developed. The genetic linkage map of 'Whangkeumbae' × 'Yali' was constructed using single nucleotide polymorphisms (SNPs) derived from Axiom Pear 70K Genotyping Array and simple sequence repeats. The integrated genetic linkage map of 'Whangkeumbae' × 'Yali' showed ~90% of genome coverage, a total genetic distance of 998.2 cM, and a marker density of 1.6 cM. F₁ progenies of 'Whangkeumbae' × 'Yali' showed normal distribution of fruit length (L), diameter (D), and L/D ratio. Three QTLs located in linkage group (LG) 6, 7, and 12 were identified with LOD thresholds of 2.8 to 3.0. Six HRM markers were developed using array-SNPs anchored in the QTLs and predicted fruit shape with 28.6 to 65.3% accuracy. Notably, accuracy was increased by ~90% using an HRM marker combination consisting of CBp06sn02, CBp07sn01, and CBp12sn03. These results could provide a better understanding of the genetic mechanism of fruit shape development and reducing pear breeding period.

Introduction

Pears included in the *Pyrus* genus are economically important fruit trees in Rosaceae family (Yamamoto and Terakami 2016). The *Pyrus* with a basic chromosome number of $x = 17$ includes four major species: *P. pyrifolia*, *P. bretschneideri*, *P. ussuriensis*, and *P. communis* (Yamamoto et al. 2014). *P. pyrifolia*, *P. bretschneideri*, and *P. ussuriensis* are distributed in East Asian countries and *P. communis* is cultivated in Europe, America, and Australia. There are diverse pear fruit shapes such as oblate, round, pyriform, and obovate. Most of the European pears represented by *P. communis* bear pyriform-shaped fruits, while Asian pears have diverse fruit shapes (White et al. 2000).

Pear fruit shape is one of the important quality indices because it affects the consumers' visual preferences. After pollination and fertilization, the receptacle of the pear begins longitudinal and horizontal development. In case of the round-shaped pears, fruit longitudinal and horizontal development proceeds simultaneously from 25 to 145 days after anthesis (Xie et al. 2013). On the other hand, during the whole fruit developmental period of pyriform pear fruit, longitudinal growth proceeded faster than horizontal growth (Bain 1961).

High-density genetic linkage maps constructed with genome-wide molecular markers are essential for genetic studies on quantitative traits (Collard et al. 2005). Pear fruit shape is also one of the quantitative traits that is affected by environmental factors (Grandillo et al. 1999; Sadrnia et al. 2007). Thus, it is possible to identify the quantitative trait loci (QTL) controlling pear fruit shape by constructing a high-density genetic linkage map.

The development of sequencing technologies has promoted genetic study in diverse plant species by allowing single nucleotide polymorphisms (SNPs) to be produced in large quantities. SNPs are the most abundant variations in plant genomes thus, they have been widely applied in plant genomic and genetic analyses. Sequencing-based technologies have been applied in the production of large numbers of SNPs, but it requires several bioinformatics analyses for genotyping. In addition, if the sequencing is not run at the same time, the SNP calling will be different for each run. However, SNP array could accurately generate millions of specific SNPs and save time and money for the genotyping of plant populations (Rasheed et al., 2017). Currently, SNP

arrays have been developed in Rosaceae family, including pear (Montanari et al. 2019), apple (Bianco et al. 2016), peach (Verde et al. 2012), and strawberry (Bassil et al. 2015).

High-resolution melting (HRM), which analyzes the temperature differences via the melting curve of PCR amplicons, can detect a single nucleotide variation. HRM analysis does not require post-PCR separation such as gel electrophoresis (Simko 2016). HRM markers have been developed based on SNPs associated with a trait of interest. For instance, Lee et al. (2012) developed HRM markers linked to genetic male sterility in pepper. Wang et al. (2016) distinguished dwarf and standard growth types of pears through HRM analysis based on SNPs associated with the *PcDw*.

The pear fruit growth process comprises two stages: cell division (stage I) and expansion period (stage II) (Bain 1961; Zhang et al. 2008). The morphological changes of pear fruit proceed rapidly during stage I, and the mature pear fruit shape can be determined before stage II. Although fruit shape is one of the target traits in pear breeding, a marker that can identify fruit shape has not been developed. Therefore, in the present study, QTL analysis was performed using a genetic linkage map and fruit shape phenotype data of immature fruits after cell division was completed in order to develop HRM markers that determine fruit shape of pears.

Materials And Methods

Plant materials and DNA extraction

The F₁ mapping population consisting of 93 progenies was derived from the interspecific cross between 'Whangkeumbae' (*P. pyrifolia*, round) × 'Yali' (*P. bretschneideri*, pyriform). 'Whangkeumbae' and the 93 F₁ progenies were grown in the orchard of Pear Research Institute, National Institute of Horticultural and Herbal Science (35°01'27.9"N, 126°44'44.5"E), in Naju, South Korea. 'Yali' was maintained in the orchard of the National Institute of Horticultural and Herbal Science (35°50'2.7"N, 127°1.8'47.3"E), in Jeonju, South Korea.

Fresh young leaves of 'Whangkeumbae', 'Yali', and the F₁ progenies were collected at full blooming stage and preserved at -70°C until DNA extraction. Total genomic DNA was extracted from the leaves of both parents and F₁ progenies. DNA was extracted with DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) according to the instruction of manufacturer. Then, the extracted DNA samples were quantified using a DS-11 Spectrophotometer (DeNovix, DE, USA) and were qualified by 1.5% agarose gel electrophoresis.

Fruit shape investigation

Immature fruits of 'Whangkeumbae', 'Yali', and their 49 F₁ progenies were collected at 93 days after full bloom (DAFB). Fruit length (L) and diameter (D) were measured using a digital vernier caliper (CD-15CP, Mitutoyo, Tokyo, Japan). L and D were measured as maximum height and width, respectively. L/D ratio was calculated. Phenotyping for fruit shape was investigated with three replications.

Pear 70K SNP array

Genotype data of 'Whangkeumbae' × 'Yali' were produced through Axiom Pear 70K Genotyping Array (Montanari et al. 2019). Raw data were subjected to several filtering steps with Axiom Analysis Suite v4.0 software. Best Practice Workflow was conducted to obtain high quality genotype data. Sample quality control (QC) thresholds

were as follow: dish quality control (DQC) ≥ 0.82 , QC call rate $\geq 97\%$, percentage of passing samples $\geq 95\%$, average call rate for passing samples $\geq 98.5\%$. SNP QC thresholds followed the default value for diploid species type.

Filtered SNPs were sorted into 6 classes: PolyHighResolution (PHR), NoMinorHomozygote (NMH), MonoHighResolution (MHR), OffTargetVariant (OTV), CallRateBelowThreshold (CRBT), and other.

SSR analysis

A total of 295 SSRs consisting of 213 pear SSRs (Yamamoto et al. 2002a, b, c; Sawamura et al. 2004; Nishitani et al. 2009; Chen et al. 2015) and 82 apple SSRs (Gianfranceschi et al. 1998; Liebhard et al. 2002) were used for the polymorphic test in 'Whangkeumbae' $\times$ 'Yali'. After the PCR amplification of the 295 SSRs in 'Whangkeumbae', 'Yali', and their F_1 individuals, PCR amplicons were confirmed by capillary electrophoresis using Fragment Analyzer (Agilent Technologies, Santa Clara, CA, USA). PROSize 3.0 software was used to analyze the capillary electrophoresis results.

Construction of genetic linkage map and QTL analysis

JoinMap 5.0 was used to construct an integrated 'Whangkeumbae' $\times$ 'Yali' map (WY map) using array-SNPs and SSRs with a pseudo-testcross mapping strategy. The SNPs and SSRs genotype data were converted to cross pollinator (CP) code and loaded into JoinMap 5.0. Among the array-SNPs, PHR type had genotype <hk $\times$ hk> and NMR type had genotype <lm $\times$ ll> and <nn $\times$ np>. Markers that showed a segregation distortion rate of more than 10% of missing data were excluded in the subsequent analysis. Grouping was performed with a logarithm of odds (LOD) threshold of 10.0. Genetic distance was calculated by Kosambi's mapping function and regression mapping.

The phenotype data and WY map were loaded onto MapQTL 6.0 to identify the QTL related to the pear fruit shape. Kruskal-Wallis test, interval mapping, and 1,000 permutation tests were carried out and the LOD threshold was decided at $p < 0.05$. After the QTL analysis, MapChart 2.3 was used to represent significant QTLs.

HRM analysis

SNPs located in fruit shape-related QTLs were converted into HRM markers. The flanking sequences (100 to 150 bp) of each SNP were obtained using SAMtools. Primer3 (<https://bioinfo.ut.ee/primer3-0.4.0/>) was used to design HRM markers. Primer pairs with a length of 18 to 23 bp, annealing temperature of 57 to 62°C, GC content of 30 to 70%, and producing 80 to 120 bp of amplicons were selected.

HRM analysis was performed using RoterGene6000 real-time cyciler (Qiagen, Hilden, Germany) with Type-it HRM PCR Kit (Qiagen, Hilden, Germany). A total of 10 μ L PCR reactant comprised 2 $\times$ HRM PCR Master Mix, including HotStarTaq Plus DNA polymerase, Type-it HRM PCR buffer with EvaGreen dye, dNTP, 10 μ M of forward and reverse primers, 30 ng of gDNA, and RNase-free water. The amplification steps were as follows: pre-denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 10 sec, annealing at 60°C for 30 sec, and extension at 72°C for 10 sec. Subsequently, fluorescence intensity was measured by increasing the temperature by 0.1°C every 2 sec in the range of 70 to 95°C. Then, the melting curve was analyzed using Rotor-Gene Q Series Software (Qiagen, Hilden, Germany).

Results

Phenotype evaluation for fruit shape

Fruit shapes of 'Whangkeumbae' and 'Yali' were round and pyriform, respectively (Fig. 1). The average L was longer in 'Yali' (5.38 ± 0.53 cm) than in 'Whangkeumbae' (4.54 ± 0.40 cm). The average L of the F_1 individuals was 5.79 ± 0.67 cm and the L distribution ranged from 4.55 to 7.31 cm. The average D for 'Whangkeumbae' and 'Yali' was 5.00 ± 0.29 and 4.20 ± 0.36 cm, respectively. The F_1 individuals displayed 3.57 to 6.95 cm of D. Based on the observed L and D, the L/D ratio of 'Whangkeumbae' and 'Yali' were 0.88 ± 0.03 and 1.30 ± 0.13 , respectively. L, D, and L/D ratio of F_1 progenies displayed normal distribution (Fig. 2). For F_1 progenies with a L/D ratio of more than 0.91, the maximum L/D ratio of 'Whangkeumbae', was considered pyriform and the remaining F_1 progenies were considered round. The number of F_1 progenies with a L/D ratio of 0.91 or higher was 4.4-folds more than that of F_1 progenies with a L/D ratio of less than 0.91.

SnP Calling And Filtering

A total of 71,363 SNPs were detected in 'Whangkeumbae' × 'Yali' after the Axiom Pear 70K SNP array. Among them, 11,200 NMH (< lm × ll > and < nn × np >) and 8,431 PHR type (< hk × hk >) SNPs were selected to construct a genetic linkage map. After filtering the SNPs showing an unexpected segregation ratio, 10,649 SNPs were finally selected for genetic linkage map construction.

Ssr Analysis

Among the 295 SSRs, 197 SSRs were polymorphic in 'Whangkeumbae' × 'Yali'. Sixty-six and 32 out of the remaining 98 SSRs were monomorphic and were not amplified, respectively. Of the 197 polymorphic SSRs, only 81 SSRs fit the CP codes. The 81 SSRs, including 28 < nn × np >, 17 < lm × ll >, 12 < hk × hk >, 18 < ef × eg >, and 6 < ab × cd > types, were used to construct a genetic linkage map.

Genetic Linkage Map Of 'whangkeumbae' × 'yali'

The WY map was divided into 17 linkage groups (LGs) corresponding to the basic chromosome number of pear (Fig. S1). The order of LG numbers corresponded with the chromosome number. The genetic linkage map consisted of 1,630 loci, including 1,570 SNPs and 60 SSRs (Table 1). The WY map had a total genetic distance of 998.2 cM. The number of markers per LG ranged from 58 to 138 and the average marker density was 1.6 cM. Each LG anchored at least one SSR, except for LG8. Based on the physical location of the anchored markers in each LG, the average chromosome coverage of the WY map was 90.5% (Table 1).

Table 1

The number of markers, genetic distance, marker density, and chromosome coverage of 'Whangkeumbae' × 'Yali' genetic linkage maps.

LG ^a	No. of SNPs	No. of SSRs	Total no. of markers	Genetic distance (cM)	Marker density (cM)	Chromosome coverage (%) ^b
1	118	2	120	52.0	2.3	76.8
2	114	6	120	61.5	2.0	99.3
3	106	5	111	60.3	1.8	98.4
4	107	8	115	55.4	2.1	90.0
5	99	3	102	52.3	2.0	73.5
6	76	2	78	55.4	1.4	94.4
7	70	3	73	63.1	1.2	95.8
8	58	0	58	56.4	1.0	95.6
9	96	1	97	55.7	1.7	97.3
10	92	1	93	58.6	1.6	95.1
11	77	1	78	63.5	1.2	97.6
12	81	3	84	57.4	1.5	95.9
13	104	6	110	61.3	1.8	87.1
14	83	7	90	55.3	1.6	94.1
15	113	5	138	80.0	1.7	97.1
16	90	6	96	60.1	1.6	78.9
17	66	1	67	49.9	1.3	71.5
Total	1,570	60	1,630	998.2		
Avg.					1.6	90.5
^a Linkage group.						
^b Calculated by dividing the physical length of LG by the full length of pseudo-chromosome.						

Fruit Shape-related Qtls In 'whangkeumbae' × 'yali'

Three candidate QTLs for L/D ratio were identified in the WY map and they were located in LG6, 7, and 12 with LOD thresholds 2.8, 3.0, and 2.9, respectively (Fig. 3). Fruit shape-related QTLs were distributed from 41.2 to 55.4 cM in LG6 and accounted for 17.8% of the explained variance. In the QTL region of LG6, 6 loci were significantly associated with L/D ratio at $p \leq 0.0001$. For QTLs in LG7 and 12, the loci ranged from 0.0 to 8.6 cM and 2.0 to 30.8 cM with an explained variance 16.5 and 23.3%, respectively. Six and 10 loci in those QTL regions

of LG7 and 12, respectively, were significantly associated with the L/D ratio ($p \leq 0.0001$). Although there were no significant QTLs related to fruit L and D, LOD graphs between the fruit L and L/D ratio were similar (Fig. 3).

Development Of Hrm Markers Associated With Fruit Shape

Six HRM markers associated with fruit shape were developed using 6 SNPs out of 22 SNPs associated with fruit shape QTLs (Table 2). Two SNPs designated by CBp06sn01 and CBp06sn02 and located in chromosome 6 had $\langle nn \times np \rangle$ and $\langle lm \times ll \rangle$ genotypes, respectively. A SNP located in chromosome 7 and designated by CBp07sn01 had $\langle lm \times ll \rangle$ genotype. Three SNPs in chromosome 12, designated by CBp12sn01, CBp12sn02, and CBp12sn03 had $\langle nn \times np \rangle$ genotype. These six HRM markers discriminated homozygous and heterozygous genotypes in 'Whangkeumbae' $\times$ 'Yali' (Fig. 4).

Table 2

Primer sequences, annealing temperature (T_m °C), amplicon size, and SNP types of six HRM markers based on fruit shape related QTLs located in linkage groups (LGs) 6, 7, and 12.

LG	Position (cM)	Marker	Primer sequences (5'-3')	T _m (°C)	Amplicon size (bp)	Genotype ^a	
						Round	Pyriform
6	50.0	CBp06sn01	For: TGGGTTGGCAGAGACATGTA	60	89	T	Y
			Rev: AGAAAAGTTTGCAGCAGACGT				
6	55.4	CBp06sn02	For: GCTCTCAAGGTTTCAACGA	60	92	K	G
			Rev: TGCAGATGAAGGTTTGGAGG				
7	0.6	CBp07sn01	For: AGCATGATATCGTTTCAGTTGAC	60	90	R	A
			Rev: GCATGCATACAGTGACAACCATA				
12	6.7	CBp12sn01	For: TTACGTATTTGGTGCAGGGG	60	93	A	R
			Rev: TGCAATCAAACAGACCCTCA				
12	25.3	CBp12sn02	For: GGATTTGAGCAACCTATTATGGC	60	120	A	R
			Rev: TCCTTTGGCCGTGACATTTG				
12	4.8	CBp12sn03	For: CTACGGCCAAATGAGCACAA	60	91	T	Y
			Rev: GAGGGAGGCTGATGGGATTT				
^a Bi-allelic variants are denoted by K (G or T), R (A or G), and Y (C or T).							

Fruit shapes of 'Whangkeumbae' × 'Yali' were divided into round and pyriform. The F_1 progenies with L/D ratio of more than 0.91 were considered pyriform and the remaining F_1 progenies were considered round. The average selection rate of the 6 HRM markers was 55.1%. Among the 6 HRM markers, CBp12sn03 had the highest selection rate (65.3%) and CBp07sn01 showed the lowest selection rate (28.6%). The selection rate for fruit shape was increased in 'Whangkeumbae' × 'Yali' using HRM marker combinations consisting of two and three markers. The HRM marker combinations composed of two markers increased the selection rate to an average of 76.6%, and the combination of CBp06sn01 with CBp12sn01, CBp06sn02 with CBp12sn03, and CBp07sn01 with CBp12sn03 showed the highest selection rate (79.6%). The HRM marker combinations consisting of three markers were able to discriminate fruit shapes with an average selection rate of 87.1%. In

particular, the selection rate of HRM marker combination consisting of CBp06sn02, CBp07sn01, and CBp12sn03 was 89.8% (Table 3).

Table 3
Selection rate of fruit shape-related 6 HRM markers in 'Whangkeumbae' × 'Yali' according to marker combination.

No. of markers	HRM marker combination	Accordance ^a	Discordance ^b	Selection rate (%)
1	CBp06sn01	30	19	61.2
	CBp06sn02	27	22	55.1
	CBp07sn01	14	35	28.6
	CBp12sn01	29	20	59.2
	CBp12sn02	30	19	61.2
	CBp12sn03	32	17	65.3
2	CBp06sn01, CBp07sn01	38	11	77.6
	CBp06sn01, CBp12sn01	39	10	79.6
	CBp06sn01, CBp12sn02	38	11	77.6
	CBp06sn01, CBp12sn03	38	11	77.6
	CBp06sn02, CBp07sn01	36	13	73.5
	CBp06sn02, CBp12sn01	37	12	75.5
	CBp06sn02, CBp12sn02	37	12	75.5
	CBp06sn02, CBp12sn03	39	10	79.6
	CBp07sn01, CBp12sn01	36	13	73.5
	CBp07sn01, CBp12sn02	36	13	73.5
	CBp07sn01, CBp12sn03	39	10	79.6
3	CBp06sn01, CBp07sn01, CBp12sn01	43	6	87.8
	CBp06sn01, CBp07sn01, CBp12sn02	42	7	85.7
	CBp06sn01, CBp07sn01, CBp12sn03	42	7	85.7
	CBp06sn02, CBp07sn01, CBp12sn01	42	7	85.7
	CBp06sn02, CBp07sn01, CBp12sn02	43	6	87.8

^aNumber of F₁ progenies showing genotype and phenotype accordance.

^bNumber of F₁ progenies showing genotype and phenotype discordance.

No. of markers	HRM marker combination	Accordance ^a	Discordance ^b	Selection rate (%)
	CBp06sn02, CBp07sn01, CBp12sn03	44	5	89.8
^a Number of F ₁ progenies showing genotype and phenotype accordance.				
^b Number of F ₁ progenies showing genotype and phenotype discordance.				

Discussion

Pear fruit shape is determined by differences in the longitudinal and horizontal development of fruit. The fruit shapes of 'Whangkeumbae' and 'Yali' were confirmed as round and pyriform, respectively, at 93 DAFB (Fig. 1). Bain (1961) reported that longitudinal growth of pyriform fruit progressed more rapidly than the round fruit during the cell division period. The average longitudinal diameter of 'Yali' was longer than the horizontal diameter, thus these results were in agreement with report of Bain (1961) and suggest that the pear fruit shape could be evaluated before the cell expansion period. In addition, phenotype distributions of L, D, and L/D ratio in 'Whangkeumbae' × 'Yali' (Fig. 2) suggest that the fruit shape is controlled by polygene (Grandillo et al. 1999; Sadrnia et al. 2007).

The resolution of WY map was increased compared to our previous 'Whangkeumbae' × 'Minibae' (WM) and 'Greensis' × 'Whasan' (GW) maps (Han et al. 2019; Oh et al. 2020) in terms of the number of mapped markers, average marker density, and genome coverage. The WM and GW maps were constructed using SNPs derived from GBS and SSRs. GBS can detect a large number of SNPs in a short time by reducing genome complexity with a restriction enzyme but, SNPs are detected in partial genome sequences (Elshire et al. 2011). The Axiom Pear 70K Genotyping Array is a powerful fixed genotyping platform for SNP screening and is more effective than GBS (Montanari et al. 2019). Indeed, Montanari et al. (2019) observed numerous polymorphic markers using the Axiom Pear 70K Genotyping Array rather than GBS in a segregating population. As a result, the genome coverage of WY map was higher than that of the previous maps constructed using GBS-SNPs.

A segregating population of 'Bayuehong' (*P. communis* × *P. bretschneideri*) × 'Dangshansuli' (*P. bretschneideri*) (BD) have been utilized for QTL analysis related to fruit characteristics including fruit shape (Zhang et al. 2013; Wu et al. 2014). Zhang et al. (2013) constructed single parental maps of 'Bayuehong' and 'Dangshansuli' using amplified fragment length polymorphism (AFLP), sequence-related amplified polymorphism (SRAP), and simple sequence repeat (SSR) markers, and detected QTLs related to L/D ratio in LG1, 2, 7, and 8 of 'Bayuehong' map. Wu et al. (2014) used SNPs detected by restriction site-associated DNA sequencing and SSRs derived from pears and apples to construct an integrated BD map and identified QTLs associated with L (LG11 and 17) and D (LG3, 11, and 17). However, three QTLs related to L/D ratio were detected in LG6, 7, and 12 of WY map (Fig. 3). Although Zhang et al. (2013) and Wu et al. (2014) used the same segregating population, the QTL analysis results were different and Wu et al. (2014) thought that the results could be affected by the resolution of genetic linkage maps. As the WY map anchored more informative array-SNPs than the reduced representation library sequencing-derived SNPs, the three QTLs related to L/D ratio in WY map could also control the fruit shape of Asian pears. Moreover, we thought that the different QTL results between the present and previous studies were

influenced by the genomic differences between 'Bayuehoung' (*P. communis* × *P. bretschneideri*) × 'Dangshansuli' (*P. bretschneideri*) and 'Whangkeumbae' (*P. pyrifolia*) × 'Yali' (*P. bretschneideri*).

Theoretically, the number of F₁ progenies for genetic study should be at least 50 (Collard et al. 2005). Although 93 F₁ progenies were used for genetic mapping, only 49 F₁ progenies were evaluated for fruit shape, as the remaining 44 F₁ progenies did not bear fruits. However, LOD values of significant QTLs (> 3.0) in LG6, 7, and 12 of the WY map supported that the 3 QTLs are associated with pear fruit shape. It was possible to overcome the relatively small number of F₁ progenies and predict the fruit shape of mature fruit as immature fruit.

Six HRM markers associated with pear fruit shape were developed, and their melting curves and normalized melting curves divided the F₁ progenies according to the marker genotypes (Figs. 4 and S2). However, several F₁ progenies showed a discrepancy between genotype and phenotype. These results suggest that the genotype of array-SNPs is correct, and the discrepancy between genotype and phenotype is due to the influence of the environment on fruit shape (Sadnia et al. 2007).

Among the 6 HRM markers, CBp07sn01 had the lowest selection rate (28.6%), suggesting that the QTL detected in LG7 of WY map is a minor QTL associated with fruit shape. The average selection rate of single markers ranged from 28.6 to 65.3%, whereas the marker combination comprised 3 markers increased the selection rate by 89.8% (Table 3). This means that all the three QTLs could influence fruit shape development in 'Whangkeumbae' × 'Yali'. The marker combination consisting of CBp06sn02, CBp07sn01, and CBp12sn03 could be used for marker-assisted selection in the pear breeding program.

Longitudinal growth of 'Yali' fruit was observed before cell expansion (Fig. 1). Although no markers had an LOD value above the LOD threshold in QTL analysis related to L, the two LOD graphs associated with L/D ratio and L were similar (Fig. 2). Notably, Zhang et al. (2013) found QTL associated with L/D ratio in the genetic linkage map of 'Bayuehong' (pyriform). Therefore, genetic factors related to pear fruit shape could control pyriform shape during the cell division period. Indeed, *ovate* cloned in tomatoes regulates cell division patterns and *suppressor of ovate* interacts with *ovate* resulting in a pyriform fruit (Wu et al. 2018). The three QTLs associated with fruit shape in 'Whangkeumbae' × 'Yali' will contribute to understanding the genetic mechanism of pear fruit shape. The HRM markers will facilitate pear breeding by selecting the desirable fruit shape early in breeding.

Declarations

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Authors' contribution

SC and SO performed the overall experiments, data analysis, and wrote the manuscript together. HH contributed to bioinformatics analysis. KK and HWJ contributed to phenotyping. DK designed and managed whole experiments and finalized the manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

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Figures

Figure 1

Fruits of ‘Whangkeumbae’ (a) and ‘Yali’ (b) collected at 93 days after full bloom. The scale bar indicates 2 cm.

Figure 2

Phenotype distribution of fruit length (a), diameter (b), and the ratio of fruit length to diameter (L/D ratio) (c) in ‘Whangkeumbae’ × ‘Yali’. The white and black inverted triangles indicate the phenotype of ‘Whangkeumbae’ and ‘Yali’, respectively.

Figure 3

Fruit shape-related QTLs in ‘Whangkeumbae’ × ‘Yali’ genetic linkage map. The scale bar on left is genetic distance (cM). Anchored markers are on the right side of each linkage group (LG). Markers black, green, and red are array-SNPs, pear SSRs, and apple SSRs, respectively. Blue bars represent QTLs associated with L/D ratio and left are logarithm of odds (LOD) values. The LOD graphs on right is for fruit length. The dotted line in LOD graphs implies a threshold ($p = 0.05$).

Figure 4

High resolution melting (HRM) results of six SNP markers. a, CBp06sn01; b, CBp06sn02; c, CBp07sn01; d, CBp12sn01; e, CBp12sn02; f, CBp12sn03. Red and blue lines are F₁ individuals having genotypes of 'Whangkeumbae' and 'Yali', respectively.

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