QTL analysis and development of HRM markers associated with fruit shape in interspecific pears (Pyrus pyrifolia × P. bretschneideri)

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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 70 K 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 2.8–3.0. Six HRM markers were developed using array-SNPs anchored in the QTLs and predicted fruit shape with 28.6–65.3% 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.

Keywords Asian pear · Genetic linkage map · Interspecific population · Molecular marker · Single nucleotide polymorphism · Quantitative trait

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 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 5-years-old 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), which corresponds to the cell expansion phase (Xie et al. 2013). Of the 93 F₁ progenies, only 49 bore fruits. 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 once with three replications.

Pear 70 K SNP array

Genotype data of ‘Whangkeumbae’ × ‘Yali’ were produced through Axiom Pear 70 K Genotyping Array (Montanari et al. 2019). Raw data were subjected to several filtering steps with Axiom Analysis Suite v4.0 software (Thermo Fisher Scientific, Inc., Waltham, MA, USA). 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 ≥ 97%, percentage of passing samples ≥ 95%, and average call rate for passing samples ≥ 98.5%. SNP QC thresholds followed the default value for diploid species type.

Using the R package, SNPolisher v1.5.2, filtered SNPs were sorted into 6 classes: PolyHigh-Resolution (PHR), NoMinorHomozygote (NMH), MonoHighResolution (MHR), OffTargetVariant (OTV), CallRateBelowThreshold (CRBT), and other. SNPs sorted into PHR (< hk × hk >) and NMH (< lm × ll > and < nn × np >) were used for linkage analysis.

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’ × ‘Yali’. After the PCR amplification of the 295 SSRs in ‘Whangkeumbae’, ‘Yali’, and their F₁ individuals, PCR amplicons were confirmed by capillary electrophoresis using Fragment Analyzer (Agilent Technologies, Santa Clara, CA, USA). PRO-Size 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’ × ‘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 × hk > and NMH type had genotype < lm × ll > and < nn × 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 1000 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 (100–150 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 18–23 bp, annealing temperature of 57–62 °C, GC content of 30–70%, and producing 80–120 bp of amplicons were selected.

HRM analysis was performed using Rotergene6000 real-time cycler (Qiagen, Hilden, Germany) with Type-it HRM PCR Kit (Qiagen, Hilden, Germany). A total of 10 µL PCR reactant comprised 2×HRM PCR Master Mix, including HotStarTaq Plus DNA polymerase, Type-it HRM PCR buffer with EvaGreen dye, dNTP, 10 µM 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 s, annealing at 60 °C for 30 s, and extension at 72 °C for 10 s. Subsequently, fluorescence intensity was measured by increasing the temperature by 0.1 °C every 2 s in the range of 70–95 °C. Then, the melting curve was analyzed using Rotor-Gene Q Series Software (Qiagen, Hilden, Germany).

HRM marker validation

The developed HRM markers discriminating fruit shape were applied to pear cultivars including round- 
(P. pyrifolia cv. Whasan and Wonhwang, P. bretschneideri cv. Dangshansuli) and pyriform-shaped cultivars (P. bretschneideri cv. Cili, Yaguangli, and Qiubaili, and P. communis cv. Bartlett, Abate Fetal, and Williams). Through the above-described DNA extraction process, DNA was extracted from young leaves of the 9 cultivars. After that, HRM analysis was repeated 2 times for each cultivar using Rotor-Gene Q Series Software (Qiagen, Hilden, Germany). ‘Whangkeumbae’ and ‘Yali’ were used as control cultivars with round and pyriform genotypes, respectively.

Results

Phenotype evaluation for fruit shape

Fruit shapes of ‘Whangkeumbae’ and ‘Yali’ were round and pyriform, respectively, at 93 DAFB (Fig. 1). The average L was longer in ‘Yali’ (5.38 ± 0.53 cm) than in ‘Whangkeumbae’ (4.54 ± 0.40 cm) at 93 DAFB. The average L of the F₁ 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 F₁ individuals displayed 3.57–6.95 cm of D. Based on the observed L and D, the L/D ratio of ‘Whangkeumbae’ and ‘Yali’ was 0.88 ± 0.03 and 1.30 ± 0.13, respectively. L, D, and L/D ratio of F₁ progenies displayed normal distribution (Fig. 2). For F₁ progenies with a L/D ratio of more than 0.91, the maximum L/D ratio of ‘Whangkeumbae’, was considered pyriform and the remaining F₁ progenies were considered round. The number of F₁ progenies with a L/D ratio of 0.91 or higher was 15.3-folds more than that of F₁ 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 70 K SNP array. Among them, 11,200 NMH (<lm<int>ll> and <nn<int>np>) and 8431 PHR type (<hk<int>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<int>np>, 17 <lm<int>ll>, 12 <hk<int>hk>, 18 <ef<int>eg>, and 6 <ab<int>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 1630 loci, including 1570 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).

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

HRM primers were designed using the flanking sequences of the 22 SNPs, but 8 primer pairs were not designed because there were no primer sequences matching the criteria. Fourteen HRM markers were analyzed and 8 of 14 HRM markers did not showed association between genotype and phenotype. Thus, 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 in chromosome 6 had \(<\text{nn×np}>\) and \(<\text{lm×ll}>\) genotypes, respectively. A SNP designated by CBp07sn01 in chromosome 7 had \(<\text{lm×ll}>\) genotype. Three SNPs designated by CBp12sn01, CBp12sn02, and CBp12sn03 in chromosome 12 had \(<\text{nn×np}>\) genotype. These six HRM markers discriminated homozygous and heterozygous genotypes in ‘Whangkeumbae’ × ‘Yali’ (Fig. 4).
Fruit shapes of ‘Whangkeumbae’ × ‘Yali’ were divided into round and pyriform. The F1 progenies with L/D ratio of more than 0.91 were considered pyriform and the remaining F1 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).

As a result of validation of the HRM marker combination in pear cultivars, CBp06sn02 discriminated ‘Yali’ and ‘Cili’. In case of CBp07sn01, all 6 cultivars displayed the same genotype as ‘Whangkeumbae’. However, the CBp12sn03 discriminated pear cultivars according to the fruit shape (Fig. S3).

**Table 1** The number of markers, genetic distance, marker density, and chromosome coverage of ‘Whangkeumbae’ × ‘Yali’ genetic linkage maps

| LG | 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                     |
| Avg| 1570        | 60          | 1630                 | 998.2                 | 1.6                 | 90.5                     |

aLinkage group
bCalculated by dividing the physical length of LG by the full length of pseudo-chromosome

**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 progresses more rapidly in pyriform fruit than the round fruit during the cell division period. The average length of ‘Yali’ was longer than the 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).
Fig. 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 ($p = 0.05$). (Color figure online)

Table 2 Primer sequences, annealing temperature (Tm, °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′) | Tm (°C) | Amplicon size (bp) | Genotypea |
|-----|---------------|--------|--------------------------|---------|-------------------|-----------|
|     |               |        | Round Pyriform            |         |                   |           |
| 6   | 50.0          | CBp06sn01 For: TGGGTGGCAGAGACATGTA Rev: AGAAAGTGGTGCAGACACGT | 60       | 89                 | T Y       |
| 6   | 55.4          | CBp06sn02 For: GCCTCTAGGTTTCAACGCA Rev: TGCAGATGAGGTTGGAGG | 60       | 92                 | K G       |
| 7   | 0.6           | CBp07sn01 For: AGCATGATATCTGTTTAGGAC Rev: GCATGATACATGTCACACCA | 60       | 90                 | R A       |
| 12  | 6.7           | CBp12sn01 For: TTACGTATTGTTGAGGGG Rev: TGCAGATCAGCAGACCTCA | 60       | 93                 | A R       |
| 12  | 25.3          | CBp12sn02 For: CTACGAGCAATTCAGACCAA Rev: GAGGGAGGCTGATGGGATT | 60       | 120                | A R       |
| 12  | 4.8           | CBp12sn03 For: GGATTTGAGCACTTTATGAGC Rev: TCTTTTGCGGCGATTTT   | 60       | 91                 | T Y       |

a Bi-allelic variants are denoted by K (G or T), R (A or G), and Y (C or T).
Fig. 4 High resolution melting (HRM) results of six SNP markers. \textbf{a} CBp06sn01; \textbf{b} CBp06sn02; \textbf{c} CBp07sn01; \textbf{d} CBp12sn01; \textbf{e} CBp12sn02; \textbf{f} CBp12sn03. Red and blue lines are F₁ individuals having genotypes of ‘Whangkeumbae’ and ‘Yali’, respectively. (Color figure online)

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 |
|                | CBp06sn02, CBp07sn01, CBp12sn03 | 44 | 5 | 89.8 |

\(^a\)Number of F₁ progenies showing genotype and phenotype accordance

\(^b\)Number of F₁ progenies showing genotype and phenotype discordance
The WY map consisted of 1,570 SNPs and 60 SSRs, which constitute 15.2% of the 10,730 markers used for linkage analysis. The remaining 84.8% markers were not mapped due to segregation distortion or physical location mismatch with closely linked markers. Nevertheless, the resolution of WY map was increased compared to our previous ‘Whangkeumbae’ × ‘Miniba’ (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 70 K 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 70 K 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 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 ‘Bayuehong’ (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. Vales et al. (2005) suggested that the number of QTLs detected in randomly selected subpopulations was higher than in selective genotype and selective phenotype subpopulations when the subpopulation size was small (n = 50). The 49 F1 progenies evaluated for fruit shape phenotype were naturally selected from the total of 93 F1 progenies, thus, it was possible to overcome the relatively small number of F1 progenies in the QTL analysis 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 (Sadrnia 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’. However, the F1 population size (49 progenies) is small to prove association between genotype and phenotype. Therefore, the HRM marker combination was applied to pear
cultivars bearing round or pyriform fruits. Interestingly, CBp12sn03 discriminates fruit shape of pear cultivars regardless of species (Fig. S3). Since the CBp06sn02 and CBp07sn01 were able to discriminate two cultivars (‘Yali’ and ‘Cili’) and one (‘Yali’) cultivar, respectively, it was thought that QTL regions related to fruit shape could be different depending on pear cultivars. Nevertheless, the CBp12sn03 could be a universal marker to discriminate two cultivars (‘Yali’ and ‘Cili’) and one cultivar (‘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.

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

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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