RESEARCH ARTICLE

Construction of a high-density genetic linkage map and QTL analysis of morphological traits in an F1 *Malusdomestica × Malus baccata* hybrid

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Abstract Apple is considered the most commonly grown fruit crop in temperate regions that brings great economic profits to fruit growers. Dwarfing rootstocks have been extensively used in apple breeding as well as commercial orchards, but the molecular and genetic basis of scion dwarfing and other morphological traits induced by them is still unclear. At present, we report a genetic map of *Malusdomestica × Malus baccata* with high density. The F_1 population was sequenced by a specific length amplified fragment (SLAF). In the genetic map, 5064 SLAF markers spanning 17 linkage groups (LG) were included. Dwarf-related and other phenotypic traits of the scion were evaluated over a 3-year growth period. Based on quantitative trait loci (QTL) evaluation of plant height and trunk diameter, two QTL clusters were found on LG 11, which exhibited remarkable influences on dwarfing of the scion. In this analysis, QTL *DW2*, which was previously reported as a locus that controls dwarfing, was confirmed. Moreover, three novel QTLs for total flower number and branching flower number were detected on LG2 and LG4, exhibited the phenotypic variation that has been explained by QTL ranging from 8.80% to 34.80%. The findings of the present study are helpful to find scion dwarfing and other *phenotypes* induced by rootstock in the apple.

Keywords Apple · Dwarfing · QTL · Genetic linkage map · SLAF-seq

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Abbreviations
SLAF   Specific length amplified fragment
PH   Plant height
TD   Trunk diameter
TF   Total flower number
IM   Interval mapping
LG   Linkage groups
QTL   Quantitative trait loci

Introduction

Apples (*Malus × domestica*, $2n = 34$), the most commonly grown ones that play a very crucial role in economic aspects because of its cultivated area of millions of hectares and production of millions of metric tons (Liu et al. 2016) are considered as fruit tree crops in temperate regions across the whole world. Scions with desirable

characteristics that are taken from apple trees and grafted onto clonal rootstocks have shown advantages in some traits, such as stress in cold hardiness, good soil anchorage, pest and disease resistance is a mature and effective method in fruit trees breeding. Perhaps the most important attribute of rootstocks is changing their ability on the growth and development of grafted scion (Gregory and George 2011). The dwarfing rootstock could greatly reduce the size and vigor of the grafted scion, shorten the juvenile phase and induce more flower buds, thus resulting in the increase of fruit yield per unit area in earlier years of the orchard (Foster et al. 2014). The dwarfing rootstock has been widely used in breeding practice and commercial orchards, while the genetic and molecular basis underlying the rootstock-induced dwarfing of the scion have been primarily explored. Although several models have been established to describe how rootstocks control dwarfing effects such as changes of hydraulic conductivity in dwarfing or semi-dwarfing rootstocks and vigorous rootstocks of fruit trees (Atkinson et al. 2003), and multiple techniques have been offered for translocation of plant hormone (Hooijdonk et al. 2010; Kamboj and Quinlan 1998; Kamboj et al. 1999), none could explain rootstocks-induced dwarfing completely, and its affect on *other morphological traits*.

Genetic linkage map, which was established from segregation of the population for an interesting trait, has become an essential tool for the discovery of quantitative trait loci (QTL) among different species (Nirubana et al. 2020; Kumar et al. 2020; Lin et al. 2019) and promoted the quality of genome assembly (Riccardo et al. 2010). Greater efforts have been made on disclosing the genetic basis of dissimilar traits, for example, disease resistance (Buti et al. 2015; Emeriewen et al. 2014; Roux et al. 2010; Tan et al. 2016), nutritional growth-related traits (Bai et al. 2012; Harrison et al. 2016; Kenis and Keulemans 2007; Moriya et al. 2015) fruit quality as well as its related traits (Longhi et al. 2013; Ntladi et al. 2018; Zhang et al. 2012) in apples via genetic mapping. Previously, the use of the next-generation sequencing approach was likely to grow billions of single nucleotide polymorphism molecular markers at one time which are more enormous in quantity and more stable than other marker types in most genomes. The traditional molecular markers, such as random amplified polymorphic DNA (RAPD), simple sequence repeat (SSR), and restriction fragment length polymorphism (RFLP), are gradually replaced by SNP in the genetic map construction (Arif et al. 2019; Shukla et al. 2021; Wang et al. 2015; Gao and Ning 2021). SLAF-seq as an optimized minimized representation library (RRL) sequencing approach, has recently been reported for completing large-scale newly SNP discovery and developing its genotypes with a high-resolution method in a single process (Sun et al. 2013).

SLAF sequencing tool has become an essential approach in constructing genetic maps of high density among various species (Niu et al. 2017; Wang et al. 2018a; Ying et al. 2018). Various genetic maps with high density for a woody plant, such as pear (*Pyrus spp*) (Long et al. 2017), mei (*Plum blossom*) (Zhang et al. 2015), sweet Osmanthus (*Osmanthus fragrans*) (He et al. 2017), Ginkgo (*Ginkgo biloba*) (Liu et al. 2017), grape (*Vitis vinifera*), (Lin et al. 2019), were successfully developed based on this method over the past few years. This method was considered in detecting the quantitative trait locus for the downy and powdery mildew resistance of grape (Lin et al. 2019), the oleic and linoleic acid content in Peanut (Hu et al. 2018), panicle length of rice (Zhu et al. 2018), downy and powdery mildew resistance in cucumber (Wang et al. 2018b), aluminum tolerance in wheat (*Triticum aestivum* l.) (Farokhzadeh et al. 2019), and fiber quality traits in cotton (Ali et al. 2018). The outcomes show that as an efficient high-throughput approach in developing polymorphic markers, SLAF-seq is an essential technique in constructing linkage maps and making QTL analyses.

Some previous studies allow the prediction of some major dwarfing loci. Rusholme Pilcher et al. (2008) firstly reported a QTL (*DW1*) at the uppermost of LG5 with a separating 'M9' × 'R5' rootstock family used, and *DW1* was strongly linked to the marker NZraAM18_700 and CH03a09. Fazio et al. (2014) executed the existence of *DW1* at the top of LG5 as well as determined a new QTL that is named *DW2* on LG11 which has a similar effect on the vigor of rootstock. Foster et al. (2015) used a rootstock population that is originated from the hybridization between 'Robusta5' and 'M9', as same as Rusholme Pilcher et al. (2008), and identified *DW1* and *DW2* again. Recently, using a M432 apple rootstock mapping population, (Harrison et al. 2016) found two QTLs, respectively, Rb1 and Rb2, accomplish to co-localize with earlier determined major dwarfing loci, *DW1* as well as *DW2*. Studies about dwarfing loci as mentioned above indicate that the chromosomal regions on both chr5 and chr11 contain valuable rootstock gene(s) controlling the vigor of the scion.

At present, a genetic map along with the high density of apple rootstock population was constructed based on SLAF-seq, which comprises 5064 SLAFs on the 17 linkage groups in the consensus linkage map. 14 QTLs for plant height, trunk diameter, total flower number, and branching flower number were detected in multiple-environment. Our finding will not only promote a further understanding of the genetic basis of apple rootstock-induced dwarfing but also provide molecular markers and/or genes that may be used in future marker-assisted selection for apple dwarfing breeding programs.

Materials and methods

Plant material or determination of phenotype

An F_1 hybridized population generated through inter-specific hybridization between 'Y-2' and 'Danxia' was used to perform QTL analysis in 2012. 'Y-2', the female parent, was selected from *Malus baccata* in the north-west of Shanxi Province in China and was often used as a dwarfed rootstock due to its impact on vigor control, shortening of time to flowering (juvenile phase), and enhancing cold resistance of the grafted scion. The male parent 'Danxia' selected from Golden Delicious was an artificial breeding cultivar, which has the characteristics of short branches and easy flowering. The hybrids were planted in the Pomology Institution, Shanxi Academy of Agricultural Sciences, China (820 m above sea level, soil pH 7.8, annual average temperature 10 °C, annual rainfall 400-600 mm, sunshine hours 2300 h/year, frost-free period 165–180 days). When the F_1 rootstocks were cultured to a height of 40 cm, 150 individuals were selected to eradicate genomic DNA from the leaves as well as the scions from an annual branch of 'Hongmantang' (a variety from a cross between 'Maypole' and *Malus baccata*) collected and grafted onto 1-year-old F_1 rootstocks in August 2013. The plants were permitted to develop for one season to strengthen the mother plant. The rootstock-induced dwarfing impacts on the development of 'Hongmantang' scions were identified. From February 2015 to 2017, plant height (PH) was estimated from the earth to the top of the crown by using a ruler, and trunk diameter (TD) was recorded at 10 cm up above the graft junction. In April 2015, total flower number (TF) on the whole tree and branching flower number (BF) were counted and trees or branches without any flowers were recorded as '0'. The phenotype statistics and Pearson correlation coefficient between each trait were performed in the software SPSS 25 (IBM, Armonk, NY, USA) and the frequency-distribution histograms were generated in Microsoft Excel.

SLAF library preparation and sequencing

The hybridized young leaves obtained from each F_1 rootstock individuals and their parents, Y-2 and Danxia, were collected and their DNA was extracted from leaves using Genomic DNA Kit (Tiangen Biotech Co., Beijing, China). At the same time, the concentration and size of DNA were estimated by electrophoresis in 0.8% agarose gel (Table S3) and by quantitating on ND-1000 spectrophotometer. A standard lambda DNA (from DE, Wilmington, NanoDrop, USA) was used to compare the size of the DNA. The size range of the DNA standard used in our study was from 125 to 23130 bp. SLAF was applied to perform high-throughput sequencing (Sun et al. 2013). In brief, the genome sequence of *Malus domestica Mill* (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?id=3750) was performed as the position genome when pre-experiment was used to computational simulation of the number of indicators created by diverse combinations of the enzyme. Afterward, the SLAF library has been developed with the help of a predestined scheme. For the F_1 population in this study, the RsaI and HaeIII (New England Biolabs, USA) combination of the enzyme was considered in digesting the genomic DNA of the parents and the F_1 population. Next, the assimilated fragments have added a single nucleotide (A) with the help of Klenow Fragment ($3 \rightarrow 5$ exo-) (NEB) and dATP at 37 C. Next, the A-tailed fragments were ligated to duplex tag-labeled sequencing adapters (PAGE-purified, Life Technologies, USA) by using T4 DNA ligase. PCR was executed by using dNTP, diluted restriction-ligation DNA samples, Q5® High-Fidelity DNA Polymerase as well as PCR primers (Forward primer: 5-AATGA-TACGGCGACCACCGA-3, reverse primer: 5-CAAGCA-GAAGACGGCATACG-3) (PAGE-purified, Life Expertise). PCR products were decontaminated through the help of Agencourt AMPure XP beads (Beckman Coulter, High Wycombe, UK) and pooled. Pooled samples were run on two percent agarose gel electrophoresis for size selection. The DNA fragmental size in the range of 264 to 314 bp without adaptors and indexes were discarded, and the remaining samples were extracted and purified using QIA quick Gel Extraction kit (Qiagen, Hilden, Germany). Finally, the purified fragments were diluted. On an Illumina HiSeq X sequencing platform (Illumina Inc., San Diego, CA, USA), paired-end genomic DNA sequencing (PE150 bp) was performed referring to the instruction by manufacturers.

Grouping and genotyping of sequence data

The SLAF-seq data processed with the procedures were illustrated (Sun et al. 2013). In the short term, poor reads with a quality score lower than 30 were discarded while the remaining pair-end reads were allocated to each progeny depending on the double barcode sequencing data. High-quality reads were mapped onto the apple genome sequence by using SOAP tool (Li et al. 2009). Sequencing reads that were mapped to the identical location have been named to a single SLAF marker (Zhang et al. 2013). Subsequently, the Single Nucleotide Polymorphism detection of each SLAF marker between parents, the SLAF markers above 3 SNPs were initially abandoned. For each SLAF marker, the alleles were explained in consonance with parental sequenced and selected reads with a sequence

depth of > 20- fold, while the depth of > tenfold has been named to alleles for F1 hybridization offspring reads. Apple is a diploid specie, one SLAF marker comprised of four genotypes, and therefore, SLAFs that alleles were above four were filtered out. Corresponding to this, SLAFs having two to four alleles were assessed as candidate SLAFs. The position distribution of selected SLAFs on the genome was described with the BLAST result by using the SOAP software. For the polymorphic SLAF markers, the marker code was examined and based on hybridization of population type.

Construction of high-density genetic map

Before the establishment of linkage map, a chi-square test (χ^2) was done to assess the Mendelian segregation proportion of one and all SLAF, and some of those significance level (above 0.05) was selected. Besides, the SLAFs with integrity of < 0.7 were excluded. Since (NGS) data may involve various genotyping errors and deletions, they may contribute to the reduction of quality of linkage maps having high density, HighMap technique (Liu et al. 2014) NGS play a crucial role in ordering SLAF markers, to correct their errors of genotypes in the linkage groups. The genetic map has been estimated based on the haplotype maps, heat maps, and synteny maps with a chromosome of every linkage group.

QTL analysis

Based on the linkage map, QTL analysis for plant height (PH), trunk diameter (TD), total flower number (TF), and branches flower number was made by using the interval mapping (IM) method via the map QTL 5.0 software (Van Ooijen 2004). The threshold of the logarithm of the odds (LOD) for the determination of a QTL was calculated through 1000 permutation tests, and the confidence interval range was set as 0.95. The name of QTL in the current study consisted of three parts. The first part was 'q' which represented a QTL. The second one was the abbreviation of each trait and the last one was the year when a trait was recorded. On the genetic linkage map, the distribution of the QTL was mapped via MapChart version 2.2(Voorrips 2002).

Results and discussion

## Phenotypic characterization of four traits in F$_1$ population

Phenotypic data were generated for four traits from a complete set of F$_1$ population (Fig. 1). The ranges of PH were 25 ~ 230 cm, 58 ~ 255 cm, and 102 ~ 290 cm, and the mean value of the PH was 142.46 ± 52.01 cm, 179.01 ± 49.40 cm, and 214.59 ± 50.26 cm in the years of 2015 to 2017 respectively (Table S2). The trunk diameter (TD) of F1 offspring's was showed continuous and significant changes from the years 2015 to 2017, while the mean value of the TD was 10.53 ± 2.88 mm, 17.26 ± 2.56 mm, and 23.77 ± 2.39 mm (Table S2) in the year of 2015 to 2017 respectively. The PH and TD of the F$_1$ population showed continuous and significant changes with the time increase, which suggests that both traits were controlled by multiple loci in F$_1$ population. The TF of the F$_1$ population was from 0 ~ 80 in 2015, and the mean value of the TF was 15.04 ± 11.83. For BF, most of the individuals had no branching flowers in 2015, because most flowers were on the trunks (Table S2). Correlation analysis showed that plant height and trunk diameter (r > 0.74, $p < 0.01$) exhibited a significantly positive correlation in 2015, 2016, and 2017 (Table 1). But the correlation value gradually decreased from 2015 to 2017. The PH and TD showed little positive correction with TF and BF (r < 0.5), and the TF had a mid level of positive correction with BF (r = 0.56, $p < 0.01$) in 2015 (Table 1).

Analysis of SLAF-seq data and genotyping

We obtained 11,420,396 paired-end reads containing 2.28 GB clean data from the parents and an average of 2,778,117 paired-end reads consisting of 554 Mb clean data for each of the 150 progenies. The Q30 ratio was 93.42 percent on average (Table 2). In the maternal line (Y-2), 183,352 SLAFs have been produced with an average of 15.57-fold coverage for each SLAF marker. In the paternal line (Danxia), in total 185,408 SLAFs have been produced with an average reportage of 16.90-fold for every SLAF marker. For the F$_1$ mapped population, we developed an average of 185,408 SLAF markers for each progeny, and the mean reportage was 6.28 fold seen as in Table 2. Finally, a total of 337,249 SLAF markers were shared by parents and all offspring were developed.

In the 337,249 shared SLAF markers, 132,722 (39.35%) were polymorphic, 204,288 (60.57%) were non-polymorphic and 239 (0.07%) were repetitive. It is found that the polymorphic SLAF marker distributed evenly on 17 chromosomes, which indicates that SLAF-seq was an effectual approach to developing polymorphic markers (Fig. 2). Among all the polymorphic SLAF markers, 36,829 were successfully genotyped with eight segregation patterns (ab × cd, aa × bb, ab × cc and cc × ab, ef × eg, hk × hk, lm × ll and nn × np) following a genotype encrypting regulation (Fig. 3). As the two parents are heterozygous varieties, 16,126 SLAF markers not included in aa × bb
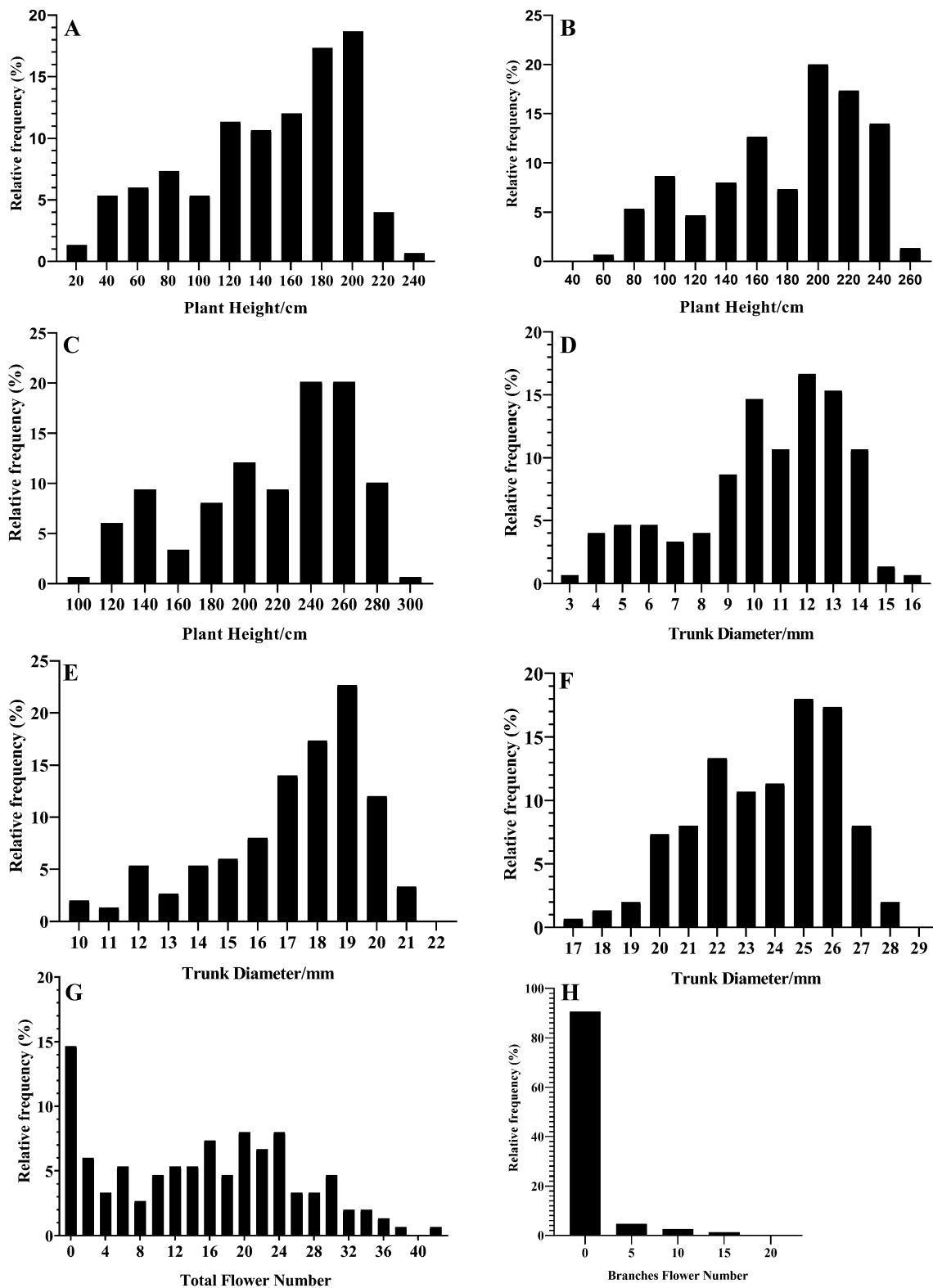
Fig. 1 Histograms of the frequency distribution of four traits for the F_1 population. **a–c**, plant height in 2015, 2016, and 2017. **d–f**, trunk diameter in 2015, 2016, and 2017. **g** Total flower number in 2015. **h** branching flower number in 2015

Table 1 Correlation analysis between each of the 4 traits across three years for the F_1 population

| Environment[a]	2015PH	2016PH	2017PH	2015TD	2016TD	2017TD	2015TF	2015BF
2015PH	1.00							
2016PH	0.99**[b]	1.00						
2017PH	0.91**	0.93**	1.00					
2015TD	0.93**	0.92**	0.87**	1.00				
2016TD	0.86**	0.84**	0.79**	0.9**	1.00			
2017TD	0.80**	0.77**	0.74**	0.83**	0.89**	1.00		
2015TF	0.48**	0.49**	0.44**	0.45**	0.35**	0.36**	1.00	
2015BF	0.17*	0.17*	0.16	0.15	0.13	0.11	0.56**	1.00

PH plant height, *TD* trunk diameter, *TF* total flower number, *BF* branches flower number

[a]2015, 2016, and 2017 represent the year when F_1 phenotype was investigated

[b]**Correlation is significant at the 0.01 level and *Correlation is significant at the 0.05 level

Table 2 Summary of SLAF-seq data for both parents as well as F_1 offspring

Sample ID	Total reads	Total bases	SLAF number	Total depth	Average depth	Q30 (%)
Paternal parent	5,601,609	1,119,931,348	183,352	2,763,044	15.07	92.43
Maternal parent	5,818,787	1,163,282,542	129,018	2,180,042	16.9	91.99
Offspring	2,778,117	554,721,926	185,408	1,165,112	6.28	93.44
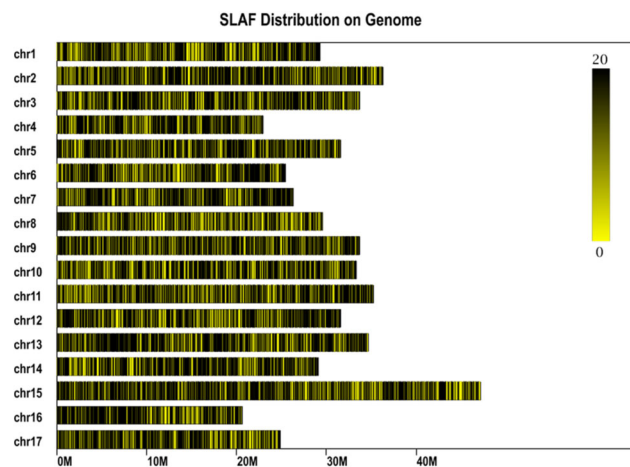
Fig. 2 Polymorphic SLAF markers on the 17 apple chromosomes. The block lines show the position of SLAF markers on the chromosomes. A legend shows the continuously changing density of SLAF markers

segregation patterns were made use of to develop a genetic linkage map.

Construction of genetic linkage map

Finally, HighMap assigned 5113 SLAF markers of high quality to the apple linkage map (Table 3). The consensus linkage map with 17 linkage groups was constructed by 5064 SLAFs. The mean sequencing depth of the SLAFs that have mapped to the linkage map was 35.63, 32.17, and 14.94 fold for the material, the paternal, and offspring, respectively. While the total genetic length of the

consensus linkage map was 1494.65 cM, with a mean marker distance of 0.36 cM between adjacent markers. The SLAF marker number assigned to the linkage group varying range of 123 in LG8 to 427 markers in LG2. LG15 was the longest LG with 303 SLAF markers spanning 115.28 cm, and the shortest linkage group was LG1 which comprises 278 SLAF markers with a 74.49 cm length. The largest gap from each linkage group in the range of 2.44 cM (LG3) to 9.16 cM (LG15). In this map, a segregation distortion ($p < 0.05$) distributed in 11 linkage groups was observedin189 (3.7%) SLAF markers. LG7 containing 112 SLAF markers presented the highest ratios of segregation distorted markers. There was no segregation of distorted SLAFs in LG1, LG2, LG3, LG6, LG12, and LG16. Besides, a pseudo-testcross strategy has contributed to the construction of the sex sex-specified genetic maps in the current result. The male genetic map harbored 2622 SLAF markers with a total genetic distance of 1666.35 cM, but in the case of the female map, it comprises 3033 SLAF markers with a total genetic distance of 1197.52 cM (Table 3). A total of 591 SLAF markers were shared by the male map as well as the female map (Table 3).

The haplotype, heat, and linearity maps were developed to estimate the aspects of this linkage map (Fig S1 and S2). The haplotype map showed the recombinant breakpoint that occurred on each chromosome of 150 F_1 offspring, in which the double exchange of the population may be considered. Most of the recombined blocks were explained distinctly (Fig S1). The showed heat map had a direct reflection of the recombining rate that existed in markers in a piece linkage group. The differences in recombination

Table 3 Characteristics of the 17 linkage groups of consensus map, male map as well as female map

Linkage Group	Consensus linkage map			Male map			Female map		
	Marker Number	Total Distance (cM)	Average Distance (cM)	Marker Number	Total Distance (cM)	Average Distance (cM)	Marker Number	Total Distance (cM)	Average Distance (cM)
1	278	74.49	0.27	134	77.99	0.58	172	70.32	0.41
2	427	96.31	0.23	272	107.76	0.4	218	83.25	0.38
3	356	78.06	0.22	218	83.87	0.38	193	68.45	0.35
4	283	79.3	0.28	157	70.85	0.45	157	77.26	0.49
5	287	84.35	0.29	84	98.22	1.17	226	69.2	0.31
6	274	87.33	0.32	163	110.82	0.68	148	57.02	0.39
7	265	88.99	0.34	153	89.44	0.58	124	68.47	0.55
8	123	98.37	0.8	66	122.57	1.86	68	66.67	0.98
9	351	84.51	0.24	211	85.99	0.41	205	80.08	0.39
10	413	103.28	0.25	191	142.5	0.75	254	63.39	0.25
11	225	77.8	0.35	176	89.47	0.51	70	32.42	0.46
12	316	75.1	0.24	187	82.34	0.44	171	67.19	0.39
13	317	87.15	0.27	142	79.83	0.56	212	82.85	0.39
14	240	66.32	0.28	130	66.13	0.51	158	64.59	0.41
15	303	115.28	0.38	121	155.77	1.29	221	70.13	0.32
16	307	105.69	0.34	131	110.45	0.84	198	94.69	0.48
17	299	92.32	0.31	86	92.35	1.07	238	81.54	0.34
Total	5,064	1494.65	–	2622	1666.35	–	3033	1197.52	–

rate were represented between any two SLAFs by cells painted with different colors in the range of yellow to purple. The interesting yellow cell indicated the lowest recombine rate, while the purple indicated the highest combination rate. And the result of heat maps that reflected SLAF markers were well-ordered in furthermost linkage groups (Fig S2). The ordered markers between each LG and the referred genome were evaluated by spearman correlation coefficient. As shown in Fig. S3, a relatively high correlation coefficient ($r > 0.90$) was observed between all LGs except LG1 ($r = 0.74$), LG7 ($r = 0.89$) and LG16 ($r = 0.64$). With all those mentioned above, a high-quality apple linkage map was constructed (Fig. 4).

Detection of QTL for phenotype data

To detect the loci that are related to four traits in the F_1 population, QTL analysis was conducted across three years (Fig. 4, Table 4). For PH, a total of 5 QTLs were identified on chromosome 11 in 2015, 2016, and 2017, respectively, and those QTLs could explain phenotype variance from 17.20% to 22.40%. Those QTLs could be clustered in the two loci with overlapped QTL intervals located closely on chromosome 11. Both *qPH15-1* and *qPH16-1* had the same QTL interval in $18.99 \sim 30.65$ cM (Cluster 1), and
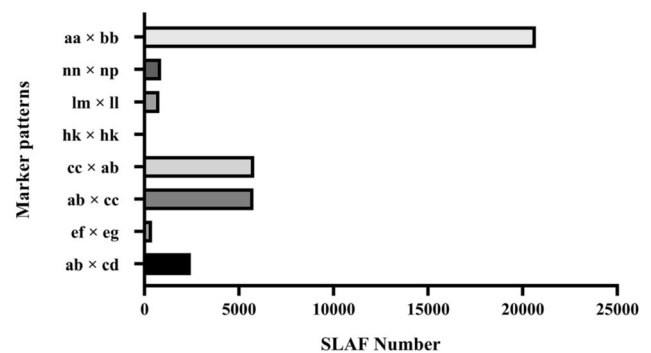
Fig. 3 The number of eight genotypes of SLAF markers. The histogram painted with different colors represents 8 segregation designs of SLAF markers. ab × cc, two parents share no allele and maternal genotype is heterozygous but paternal genotype is homozygous. cc × ab, two parents share no allele and maternal genotype is homozygous but paternal genotype is heterozygous. ef × eg, two parents share one allele, and maternal and paternal genotype both are heterozygous. hk × hk, two parents have the same allele and maternal and paternal genotype both are heterozygous. lm × ll, two parents share one allele, and maternal genotype is heterozygous but paternal genotype is homozygous. nn × np, two parents share one allele, and maternal and maternal genotype is homozygous but paternal genotype is heterozygous. aa × bb, two parents share no allele, and maternal and paternal genotype both are homozygous

qPH15-2, *qPH16-2,* and *qPH17* were mapped in $31.84 \sim 46.13$ cM (Cluster 2). For TD, 6 QTLs were also
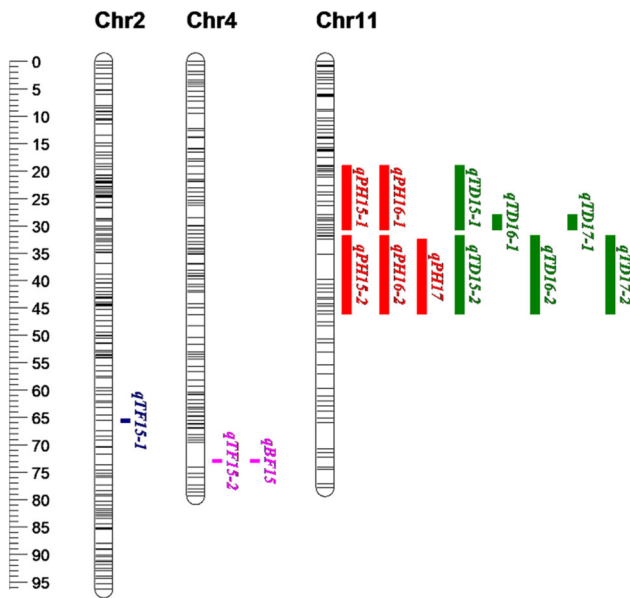
Fig. 4 The position of QTL for PH, TD, TF, and BF on different chromosomes in a F$_1$ population derived from the cross Y-2 × Danxia across 3 years. The bars located on the right of LG show the QTL identified for PH (red), TD (green), TF (blue), and BF (pink). The QTL's name is the right of QTL bar

correlation in phenotype data. For TF, two QTLs (*qTF15-1* and *qTF15-2*) were detected on chromosomes 2 and 4, respectively, which could explain a total of 8.80% and 9.20% of phenotypic variance in 2015. A QTL, *qBF15*, was detected on chromosome 4 in 2015, and this can explain 34.80% of phenotypic variance. Both *qTF15-2* and *qBF15* were mapped into the same QTL interval on chromosome 4.

The SLAF-seq approach, combined with high-throughput sequencing and locus-specific amplification, has been reported to an efficient solution for de novo SNP identifications and large-scale genotyping (Sun et al. 2013). The SLAF-seq method improves the efficiency of reduced representation library (RRL) by performing a SLAF destined experiment, optimizing the procedures to amplify as well as a selection of size, developing a double barcode system as well as a quality score algorithm to assess the value of genotyping and SNP innovation (Sun et al. 2013). SLAF sequencing can generate large amounts of sequence information and handle whole genome density distributions, which ensures density, uniformity, and efficiency of marker development that insist researcher to use this method for several crops, such as sesame, soybean, oil rape, and other vegetables. crops. In the present study, the enzyme combination, RsaI + HaeIII, based on a destined scheme of the apple genome information, was contributed to digesting of genomic DNA. Further, fragments with a length range of 264–314 bp have been selected in a pilot experiment for paired-end sequencing, which was provided the uniformity, density as well as efficacy of marker

determined on chromosome 11 across 3 years with PVE value from 14.00% to 20.80%. All of *qTD15-1*, *qTD16-1*, and *qTD17-1* were mapped into 18.99 ∼ 30.65 cM, which were located in Clusters 1, and *qTD15-2*, *qTD16-2* and *qTD17-2* mapped into 31.83 ∼ 46.13 cM were located in Clusters 2. The co-location of QTL for PH and TD confirmed the fact that PH and TD had a significant positive

Table 4 QTL identified for PH, TD, TF and BF in the F$_1$ population from 2015 to 2017

Trait	Year	QTL name	LG[a]	QTL interval (cM)	Max LOD[b]	PVE[c] (%)
Plant height	2015	*qPH15-1*	11	18.99 ∼ 30.65	6.54	18.20
	2015	*qPH15-2*	11	31.84 ∼ 46.13	7.81	21.30
	2016	*qPH16-1*	11	18.99 ∼ 30.65	6.15	17.20
	2016	*qPH16-2*	11	31.84 ∼ 46.13	7.36	20.20
	2017	*qPH17*	11	32.41 ∼ 46.13	8.26	22.40
Trunk diameter	2015	*qTD15-1*	11	18.99 ∼ 30.65	6.55	18.20
	2015	*qTD15-2*	11	31.83 ∼ 46.13	7.61	20.80
	2016	*qTD16-1*	11	27.87 ∼ 30.65	6.16	17.20
	2016	*qTD16-2*	11	31.83 ∼ 46.13	6.94	19.20
	2017	*qTD17-1*	11	27.87 ∼ 30.65	4.91	14.00
	2017	*qTD17-2*	11	31.83 ∼ 46.13	5.24	14.90
Total Flower Number	2015	*qTF15-1*	2	65.59 ∼ 65.59	3.01	8.80
	2015	*qTF15-2*	4	75.93 ∼ 75.93	3.13	9.20
Branches Flower Number	2015	*qBF15*	4	75.93 ∼ 75.93	13.92	34.80

[a] Linkage group

[b] The maximum LOD of the marker in QTL interval

[c] Phenotypic variance explained by the QTL

enlargement. Subsequently, 337,249 high-grade SLAFs were developed, and 132,722 (39.35%) of these were polymorphic. Polymorphic SLAFs comprised of 5064 were used to construct the linkage map, in both parents and the offspring being 33.90-fold and 14.94-fold, respectively. These data distinctly showed that the SLAF method is a highly effective method for both markers genotyping as well as the fast production of a high-density genetic map on large scale.

Previous studies have identified multiple major dwarfing loci by mapping different populations. For instance, Rusholme Pilcher et al. (2008) first reported a QTL (DW1) at the top of LG5 with the use of a segregating 'M9' × 'R5' rootstock family and DW1 was strongly linked to the marker NZraAM18_700 and CH03a09. Fazio et al. (2014) conducted a QTL analysis by using "O3R5" rootstock family and "G935B9" self-rooted family that confirmed the existence of DW1 at the top of LG5 proved before determining a new QTL named DW2 (LOD = 6.4) on LG11 which has a corresponding impact on vigor. Besides, DW1 on LG 5 and DW2 on LG11 were also identified by using a rootstock population that was derived from the crossing between 'Robusta5'and 'M9' (Foster et al. 2015), these results showed that the combination of DW1 and DW2 had a strong influence on dwarfing induced by rootstock, and DW1 had a stronger effect compare toDW2. Recently, Harrison et al. (2016) was identified 3 QTLs for root bark percentage in the M432 mapping population, two of which, Rb1 and Rb2, have been established to co-localize with the foremost dwarfing loci previously screened, DW1 and DW2. Thus, the dwarf loci as mentioned above indicated that the chromosomal regions on chr5 and chr11 comprise of valuable rootstock genes. In the current study, QTL analysis was executed for plant height and trunk diameter in the "Danxia × Y-2" mapping population, and two major dwarfing QTL clusters were screened in the genetic region of 18.99 ∼ 30.65 cM and 31.84 ∼ 46.13 cM on LG11, which were located in the region of 6.92 to 26.2 Mb of chromosome 11 and had an overlapped region with DW2 that reported earlier by Fazio et al. (2014). Interestingly, three novel QTLs for total flower number and branching flower number were identified on LG2 and LG4, displayed the phenotypic variation explained by QTL ranging from 8.80% to 34.80%, that probably induced by scion dwarfing. Compared with the previous studies, we did not map any DW1 locus on the LG5. The possible reason for differential result in our present study could be because of using a new dwarfing rootstock "Y-2" that was carefully selected few years ago from "Shandingzi" (Malus baccata) resource to develop the mapping population. Whereas, previous studies were

utilized different apple germplasm resources to reveal the genetic basis of rootstock-induced dwarfing and 'Malling' series of apple rootstocks were used as parents to generate populations in these studies (Foster et al. 2015; Harrison et al. 2016; Pilcher et al. 2008). Other reasons could be the dissimilarities in population makeup and its size, the genotype of the scion(s) grafted to rootstock populations (or left ungrafted), the growth circumstances and the traits analyzed as well as the duration time of phenotyping (Foster et al. 2015).

Conclusions

Our present study was intended to generate a high-density linkage map based on SLAF-seq and construct the QTL analysis for dwarfing traits of Malus domestica × Malus baccata F_1 population. QTL for plant height, trunk diameter, total flower number, and branching flower number were identified in the F_1 population from 2015 to 2017. Two QTL clusters were identified on chromosome 11 have a significant influence on scion dwarfing, which was previously reported as controlling dwarfing locus DW2. Besides, three novel QTLs for total flower number and branching flower number were identified on LG2 and LG4. Therefore, these findings suggested the presence of genes influencing the rootstock-induced dwarfing and that would help the breeder to find scion dwarfing induced by rootstock in the apple.

Author contributions TZ Yang planned and designed the research.HC Cai collected the plant materials, performed the experiment, analyzed the data, interpreted results, and wrote the manuscript. Q Wang and JD Gao contributed to sample data collection. CY Li analyzed the part of data. XM Du and BP Ding provided suggestions in drafting the manuscript and edited the manuscript. All the authors have read and approved the final manuscript.

Availability of data and materials All datasets during the above manuscript are available from the additional file or corresponding author.

Declarations

Conflict of interest The authors have declared that they have no competing interests.

Human and animal rights This article had no experiments with human participants or animals.

References

Ali I, Teng Z, Bai Y et al (2018) A high density SLAF-SNP genetic map and QTL detection for fiber quality traits in *Gossypium hirsutum*. BMC Genomics 19

Arif R, Lee S, Shahid M et al (2019) Genotyping of ribosomal proteins in Sordaria fimicola to study genetic polymorphisms and phosphorylation modifications. Intl J Agric Biol 22:247–252

Atkinson C, Dover C, Taylor L et al (2003) Root and stem hydraulic conductivity as determinants of growth potential in grafted trees of apple (Malus pumila Mill.). J Exp Bot 54:1221–1229

Bai T, Zhu Y, Fernández-Fernández F et al (2012) Fine genetic mapping of the Co locus controlling columnar growth habit in apple. Mol General Genetics 287:437–450

Buti M, Poles L, Caset D et al (2015) Identification and validation of a QTL influencing bitter pit symptoms in apple (Malus × domestica). Mol Breeding 35:1–11

Emeriewen O, Richter K, Kilian A et al (2014) Identification of a major quantitative trait locus for resistance to fire blight in the wild apple species Malus fusca. Mol Breeding 34:407–419

Farokhzadeh S, Fakheri B, Nezhad N et al (2019) Mapping QTLs of flag leaf morphological and physiological traits related to aluminum tolerance in wheat (Triticum aestivum L.). Physiol Mol Biol Plants. 25:975–990

Fazio G, Wan Y, Kviklys D et al (2014) Dw2, a new dwarfing locus in apple rootstocks and its relationship to induction of early bearing in apple scions. J Am Soc Hortic Sci 139:87–98

Foster T, Watson A, van Hooijdonk B et al (2014) Key flowering genes including FT-like genes are upregulated in the vasculature of apple dwarfing rootstocks. Tree Genet Genomes 10:189–202

Foster T, Celton J, Chagné D et al (2015) Two quantitative trait loci, Dw1 and Dw2, are primarily responsible for rootstock-induced dwarfing in apple. Hortic Res 2:15001–15001

Gao J, Ning X (2021) SNP detection and population structure evaluation of *Salix gordejevii* Y. L. Chang et Skv. in Hunshandake Sandland, Inner Mongolia. China. Physiol Mol Biol Plants 27:997–1005

Gregory P, George T (2011) Feeding nine billion: the challenge to sustainable crop production. J Exp Bot 62:5233–5239

Harrison N, Harrison R, Barber-Perez N et al (2016) A new three-locus model for rootstock-induced dwarfing in apple revealed by genetic mapping of root bark percentage. J Exp Bot 67:1871–1881

He Y, Yuan W, Dong M et al (2017) The first genetic map in sweet Osmanthus (Osmanthus fragransLour.) using specific locus amplified fragment sequencing. Front Plant Sci 8:1621

Hooijdonk B, Woolley D, Warrington I et al (2010) Initial alteration of scion architecture by dwarfing apple rootstocks may involve shoot-root-shoot signaling by auxin, gibberellin, and cytokinin. J Hortic Sci Biotechnol 85:59–65

Hu X, Zhang S, Miao H et al (2018) High-Density Genetic Map Construction and Identification of QTLs Controlling Oleic and Linoleic Acid in Peanut using SLAF-seq and SSRs. Sci Rep 8:5479

Kamboj J, Quinlan J (1998) The apple rootstock and its influence on endogenous hormones. Acta Hort 463:143–152

Kamboj J, Ast P, Baker J (1999) Identification and quantitation by GC-MS of zeatin and zeatin riboside in xylem sap from rootstock and scion of grafted apple trees. Plant Growth Regul 28:199–205

Kenis K, Keulemans J (2007) Study of tree architecture of apple (Malus × domestica Borkh.) by QTL analysis of growth traits. Mol Breeding 19:193–208

Kumar A, Saripalli G, Jan I et al (2020) Meta-QTL analysis and identification of candidate genes for drought tolerance in bread wheat (Triticum aestivum L.). Physiol Mol Biol Plants 26:1713–1725

Li R, Yu C, Li Y et al (2009) SOAP2: an improved ultrafast tool for short read alignment. Bioinformatics 25:1966–1967

Lin H, Leng H, Guo Y et al (2019) QTLs and candidate genes for downy mildew resistance conferred by interspecific grape (V. vinifera L. × V. amurensis Rupr.) crossing. Sci Hortic 244:200–207

Liu D, Ma C, Hong W et al (2014) Construction and analysis of high-density linkage map using high-throughput sequencing data. PloS One 9:e98855

Liu Z, Bao D, Liu D et al (2016) Construction of a genetic linkage map and QTL analysis of fruit-related traits in an F1 Red Fuji x Hongrou apple hybrid. Open Life Sci 11:487–497

Liu H, Cao F, Yin T et al (2017) A highly dense genetic map for *Ginkgo biloba* constructed using sequence-based markers. Front Plant Sci 8:1041

Long W, Li X, Lei W et al (2017) Construction of a high-density genetic linkage map in pear (*Pyrus communis × Pyrus pyrifolia nakai*) using SSRs and SNPs developed by SLAF-seq. Sci Hortic 218:198–204

Longhi S, Hamblin M, Trainotti L et al (2013) A candidate gene based approach validates Md-PG1 as the main responsible for a QTL impacting fruit texture in apple (Malus x domestica Borkh). BMC Plant Biol 13:37

Moriya S, Iwanami H, Haji T et al (2015) Identification and genetic characterization of a quantitative trait locus for adventitious rooting from apple hardwood cuttings. Tree Genet Genomes 11:59

Nirubana V, Vanniarajan C, Aananthi N et al (2020) Screening tolerance to phosphorus starvation and haplotype analysis using phosphorus uptake 1 (Pup1) QTL linked markers in rice genotypes. Physiol Mol Biol Plants 26:2355–2369

Niu D, Du Y, Wang Z et al (2017) Construction of the first high-density genetic linkage map and analysis of quantitative trait loci for growth-related traits in *Sinonovacula constricta*. Mar Biotechnol 19:488–496

Ntladi S, Human J, Bester C et al (2018) Quantitative trait loci (QTL) mapping of blush skin and flowering time in a European pear (Pyrus communis) progeny of "Flamingo" × "Abate Fetel". Tree Genet Genomes 14:1–24

Pilcher R, Celton J, Gardiner S (2008) Genetic markers linked to the dwarfing trait of apple rootstock "Malling 9". J Am Soc Hortic Sci 133:100–106

Riccardo V, Andrey Z, Jason A et al (2010) The genome of the domesticated apple (Malus × domestica Borkh.). Nat Genet 42:833–839

Roux L, Khan M, Broggini G et al (2010) Mapping of quantitative trait loci for fire blight resistance in the apple cultivars "Florina" and "Nova Easygro". Genome 53:710–722

Shukla R, Tiwari G, Joshi B et al (2021) GBS-SNP and SSR based genetic mapping and QTL analysis for drought tolerance in upland cotton. Physiol Mol Biol Plants 27:1731–1745

Sun X, Liu D, Zhang X et al (2013) SLAF-seq: an efficient method of large-scale de novo SNP discovery and genotyping using high-throughput sequencing. PloS One 8:e58700

Tan Y, Lv S, Liu X et al (2016) Development of high-density interspecific genetic maps for the identification of QTLs conferring resistance to *Valsa ceratosperma* in apple. Euphytica 213:10

Van Ooijen JW (2004) MapQTL 5, Software for the mapping of quantitative trait loci in experimental populations. Kyazma B V, Wageningen, Netherlands

Voorrips R (2002) MapChart: software for the graphical presentation of linkage maps and QTLs. J Hered 93:77–78

Wang S, Chen J, Zhang W et al (2015) Sequence-based ultra-dense genetic and physical maps reveal structural variations of allopolyploid cotton genomes. Genome Biol 16:108

Wang Y, Tan J, Wu Z et al (2018a) STAYGREEN, STAY HEALTHY: a loss-of-susceptibility mutation in the *STAY-GREEN* gene provides durable, broad-spectrum disease resistances for over 50 years of US cucumber production. New Phytologist 221

Wang Y, Vandenlangenberg K, Wen C et al (2018b) QTL mapping of downy and powdery mildew resistances in PI 197088 cucumber with genotyping-by-sequencing in RIL population. Theor Appl Genet 131:597–611

Ying J, Ma M, Bai C et al (2018) TGW3, a major QTL that negatively modulates grain length and weight in rice. Mol Plant 11:750–753

Zhang Q, Ma B, Hui L et al (2012) Identification, characterization, and utilization of genome-wide simple sequence repeats to identify a QTL for acidity in apple. BMC Genom 13

Zhang Y, Wang L, Xin H et al (2013) Construction of a high-density genetic map for sesame based on large scale marker development by specific length amplified fragment (SLAF) sequencing. BMC Plant Biol 13:141–141

Zhang J, Zhang Q, Cheng T et al (2015) High-density genetic map construction and identification of a locus controlling weeping trait in an ornamental woody plant (*Prunus mume* Sieb. et Zucc). DNA Res 22:183

Zhu Z, Li X, Wei Y et al (2018) Identification of a novel QTL for panicle length from wild rice (*Oryza minuta*) by specific locus amplified fragment sequencing and high density genetic mapping. Front Plant Sci 9:1492

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