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RESEARCH ARTICLE

High density genetic map and quantitative trait loci (QTLs) associated with petal number and flower diameter identified in tetraploid rose

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Abstract

Rose is one of the most important ornamental and economic plants in the world. Modern rose cultivars are primarily tetraploid, and during meiosis, they may exhibit double reduction or preferential chromosome pairing. Therefore, the construction of a high density genetic map of tetraploid rose is both challenging and instructive. In this study, a tetraploid rose population was used to conduct a genetic analysis using genome sequencing. A total of 17 382 single nucleotide polymorphism (SNP) markers were selected from 2 308 042 detected SNPs. Combined with 440 previously developed simple sequence repeats (SSR) and amplified fragment length polymorphism (AFLP) markers, a marker dosage of 6 885 high quality markers was successfully assigned by GATK software in the tetraploid model. These markers were used in the construction of a high density genetic map, containing the expected seven linkage groups with 6 842 markers, a total map length of 1 158.9 cM, and an average inter-marker distance of 0.18 cM. Quantitative trait locus (QTL) analysis was subsequently performed to characterize the genetic architecture of petal number and flower diameter. One major QTL (qpnum-3-1) was detected for petal number in three consecutive years, which explained 20.18–22.11% of the variation in petal number. Four QTLs were detected for flower diameter; the main locus, qfdia-2-2, was identified in two consecutive years. Our results will benefit the molecular marker-assisted breeding of modern rose cultivars. In addition, this study provides a guide for the genetic and QTL analysis of autotetraploid plants using sequencing-based genotyping methods.

Keywords: rose, tetraploid, genetic linkage map, QTLs

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1. Introduction

Rosa L. is an important genus in the Rosaceae family, which has a long history of cultivation, as well as a vast area of origin, abundant morphological variation, and strong adaptability. In view of its high economic and ornamental value, rose has been a worldwide focus of

breeding and research. Traditional breeding methods can produce unpredictable results, but molecular breeding can significantly improve the breeding efficiency and offers the potential to accelerate the breeding process (Smulders *et al*. 2019).

The construction of a genetic map is a foundation that provides guidance for many downstream studies, such as accurate gene location, cloning, functional gene structure analysis, and functional verification. Since the 1990s, with the development of DNA molecular marker technology, genetic mapping has been performed in both diploid and tetraploid populations of *Rosa*. Previous mapping at the diploid level has concentrated on a number of specific populations: 1) The 94/1 and 97/7 populations obtained by crossing different individuals of wild and modern roses (Debener and Mattiesch 1999; Linde and Debener 2003; Yan *et al*. 2005; Linde *et al*. 2006). 2) A series of populations using *R. wichuriana* as parents, including an Hw population (Crespel *et al*. 2002), a population from Spain (Dugo *et al*. 2005), and a WOB population (Shupert *et al*. 2007). After map integration using these diploid populations, a series of traits, including petal number, flowering period, and continuous flowering, were initially located in a specific chromosome region (Spiller *et al*. 2011). 3) An *R. roxburghii* population (Xu *et al*. 2005), which was primarily used to study the genetic mechanism of powdery mildew resistance.

At the tetraploid level, mapping populations that had previously been developed include: 1) The 90-69 F_2 population (Rajapakse *et al*. 2001; Zhang *et al*. 2006). 2) The 95/1, 95/2, and 95/3 populations for black spot resistance (Malek *et al*. 2000; Yan *et al*. 2006). 3) The GGFC population which was derived from the hybridization of the tetraploid rose cultivar Golden Gate and Fragrant Cloud, to study the inheritance of flower fragrance and vase life (Guterman *et al*. 2002; Gar *et al*. 2011). 4) The K5 population, which was derived from the hybridization of tetraploid rose cultivars, was used to study the density of spines, petal number, resistance to powdery mildew, and anthocyanin metabolism (Koning-Boucoiran *et al*. 2012, 2015; Carvalho *et al*. 2015; Gitonga *et al*. 2016; Bourke *et al*. 2018a). 5) The YS population, derived from the hybridization of the tetraploid modern rose cultivar 'Sun City' and the ancient Chinese rose cultivar 'Yunzheng Xiawei', which segregates for a number of traits, such as petal number, flower size, and flower color (Yu *et al*. 2014).

More recently, the construction of high-density genetic maps has been based on single nucleotide polymorphism (SNP) array technology and genotyping-by-sequencing (GBS) (Vukosavljev *et al*. 2016; Bourke *et al*. 2017; Yan *et al*. 2018). Next-generation sequencing (NGS)-based technologies, such as restriction site-associated DNA sequencing (RAD-seq) and specific-locus amplified fragment sequencing (SLAF-seq), have emerged as powerful techniques that provide high resolution for large-scale genotyping in quantitative trait locus (QTL) mapping or gene discovery studies. In autopolyploid plants, SNP arrays are still more widely used because of their simpler data analysis and allegedly higher accuracy, although sequencing-based genotyping is rapidly closing this gap. Using software, such as the fitTetra package in R, the fluorescent signal of SNP arrays can be converted into marker allele dosages (Voorrips *et al*. 2011), enabling subsequent genetic linkage analysis to be conducted using specialized polyploid mapping software. SNP detection using SNP arrays has been conducted in several autopolyploids, including *Medicago sativa* (Li *et al*. 2014), *Chrysanthemum* (van Geest *et al*. 2017), rose (Koning-Boucoiran *et al*. 2015), and tetraploid sour cherry (Peace *et al*. 2012). However, the cost of array development is relatively high, and it is difficult to detect novel polymorphisms in more diverse germplasm using this technology, which is also called "ascertainment bias" (Lachance and Tishkoff 2013). An objective comparison of the costs between different technologies is challenging, although it has been suggested that both array and sequencing-based approaches to genotyping may have comparable costs when all the associated processing steps have been factored in (Bajgain *et al*. 2016).

In contrast, directly sequencing the germplasm of interest is not restricted to a predefined set of SNP loci, making it an attractive approach for the analysis of autopolyploids. However, challenges remain in the application of this technology. In addition, this method can only reliably distinguish different types of heterozygotes, such as Aaaa, AAaa, and AAAa, when the depth of sequencing is 60× (UitdeWilligen *et al*. 2013), which is cost-prohibitive, and restricts the application of this genotyping method.

In this study, the tetraploid *Rosa* YS population was used to identify polymorphisms by reduced-representation genome sequencing using SLAF-seq. The aim of this study was to explore the use of a tetraploid model for genotyping and genetic linkage analysis in the construction of a high density genetic map of tetraploid rose and to analyze the petal number and flower size for QTLs. We compared different filtering and genotyping approaches to optimize map quality in a complex polyploid species and provided insight for a more detailed genetic dissection of important morphological traits in *Rosa hybrida* L. in the future.

2. Materials and methods

2.1. Experimental materials

The female parent 'Yunzheng Xiawei' (YX) is a traditional tetraploid Chinese rose cultivar with pinkish-white to

white flowers, and the male parent 'Sun City' (SC) is a modern tetraploid rose cultivar with pure yellow flowers. The tetraploid YS population has been previously used to construct a genetic linkage map using AFLP and SSR markers (Yu *et al*. 2014). Both parents and the resulting F_1 population (187 individuals) were planted at the Xiaotangshan Experimental Base of the National Engineering Research Center for Floriculture, Beijing, China. The parents and the YS population were grown in the same environment.

2.2. DNA extraction and genotyping

Total DNA was extracted using a plant genomic DNA extraction kit from Tiangen (Tiangen Biotech, Beijing, China). The DNA was extracted from young leaves of the YS population and their parents as described by Yu (2015), with minor modifications. DNA was extracted from pooled samples of three biological replicates per individual. We collected at least three leaves and mixed the samples to extract the total DNA for each individual.

SLAF-seq was used to genotype parents and 187 F_1 individuals as described by Sun *et al*. (2013). The genome of *R. chinensis* Old Blush (Hibrand Saint-Oyant *et al*. 2018) was used as the reference genome. A preliminary experiment was conducted to estimate the restriction enzymes and conditions required to obtain high quality SLAFs. *Rsa*I and *Eco*RV were used to digest the genomic DNA of the YS population after the evaluation. An A-overhang was subsequently added to the digested fragments followed by the addition of Klenow Fragments (New England Biolabs, Beverly, MA, USA) and dATP at 37°C. The polyacrylamide gel electrophoresis (PAGE)-purified duplex tag-labeled sequencing adapters were then ligated to the A-tailed DNA using T4 DNA ligase (New England Biolabs). After the polymerase chain reaction (PCR) and electrophoresis, the purified products were sequenced by an Illumina HiSeq 2500 System (Illumina, San Diego, CA, USA) at the Biomarker Technologies Corporation (Beijing, China).

2.3. Analysis and genotyping of sequencing data

The raw sequence data were filtered as follows: (1) the adapter sequences were removed from the reads; (2) the bases with a quality score <20 or with an N at the 5′ end were omitted; (3) the bases with a sequence quality score <3 or with an N at the 3′ end were omitted; (4) to perform sliding window trimming, the size of the window was set to 4 bp, and the bases with a quality score <20 were deleted (Bolger *et al*. 2014); (5) the reads with more than 10% N were removed; (6) reads with more than 40% bases with a quality score <15 were omitted; (7) reads with a length <30 bp after removing the adapter sequences and mass pruning were removed.

The filtered data were mapped onto the reference genome sequence (Hibrand Saint-Oyant *et al*. 2018) using the MEM method in BWA Software (Li and Durbin 2009). The BAM files were corrected following GATK Best Practices, and SNPs were detected with the Haplotype Caller method in GATK (McKenna *et al*. 2010).

SNP markers with an average sequencing depth greater than 10× or 20× were filtered in both parents and offspring, resulting in two datasets for later comparison purposes. Genotype calling of the filtered SNP markers was performed by both GATK and updog software under the autotetraploid mode, which generated nine distinct bi-allelic marker segregation types, namely, 1×0, 0×1, 2×0, 0×2, 1×1, 1×2, 2×1, 1×3 and 2×2 (Table 1) (Bourke *et al*. 2016; Gerard *et al*. 2018).

2.4. Linkage map construction of tetraploid rose

Linkage map construction was performed using the polyploid mapping software polymapR (Bourke *et al*. 2018b) with three types of molecular markers, namely, high-quality SNPs obtained by sequencing, pre-developed simple sequence repeats (SSRs), and amplified fragment length polymorphisms (AFLPs) (Yu 2015).

Table 1 Nine segregation types in an autotetraploid population

Type	Paternal genotype	Maternal genotype	Separation ratio of offspring genotypes				
			aaaa	Aaaa	AAaa	AAAa	AAAA
			0	1	2	3	4
1×0	Aaaa	aaaa	1/2	1/2	0	0	0
0×1	aaaa	Aaaa	1/2	1/2	0	0	0
2×0	AAaa	aaaa	1/6	4/6	1/6	0	0
0×2	aaaa	AAaa	1/6	4/6	1/6	0	0
1×1	Aaaa	Aaaa	1/4	2/4	1/4	0	0
1×2	Aaaa	AAaa	1/12	5/12	5/12	1/12	0
2×1	AAaa	Aaaa	1/12	5/12	5/12	1/12	0
1×3	Aaaa	AAAa	0	1/4	2/4	1/4	0
2×2	AAaa	AAaa	1/36	8/36	18/36	8/36	1/36

The dosage-scored marker data were imported into polymapR, and three types of markers were further filtered. The filtration criteria used in this study were as follows: (1) the markers deviating from the expected segregation ratios under the polysomic model were filtered out (P<0.01), and (2) the markers and individuals with more than 10% missing data were filtered out. Markers with a single dose (1×0) were clustered into different linkage groups (LGs) with a logarithm of odds (LOD) score of 5. Subsequently, the selected high quality markers were ordered using the multi-dimensional scaling algorithm as implemented in the MDSmap package (Preedy and Hackett 2016). Finally, integrated genetic maps and marker dosages were used to generate phased linkage maps, which were needed for subsequent QTL analysis.

2.5. Analysis of phenotypic traits

The floral traits of YS population were shown in Fig. 1. Three randomly selected flowers were harvested at the full bloom stage from the YS population and their parents in May 2015 and 2016. The flower diameter was measured immediately with a Vernier caliper, and the number of petals was counted. Each flower was measured three times, and the average was taken as the diameter of a single flower. The petalized stamens were also included when the number of petals was counted. The number of petals was counted for three consecutive years from 2014 to 2016, and the size of the flowers was measured from 2015 to 2016. Descriptive statistics of flower diameter and petal number, and histograms of their frequency distributions, were generated using SPSS (R23.0.0.0) (IBM, Inc., Armonk, NY, USA).

2.6. QTL analysis

QTL analysis was conducted using interval mapping.

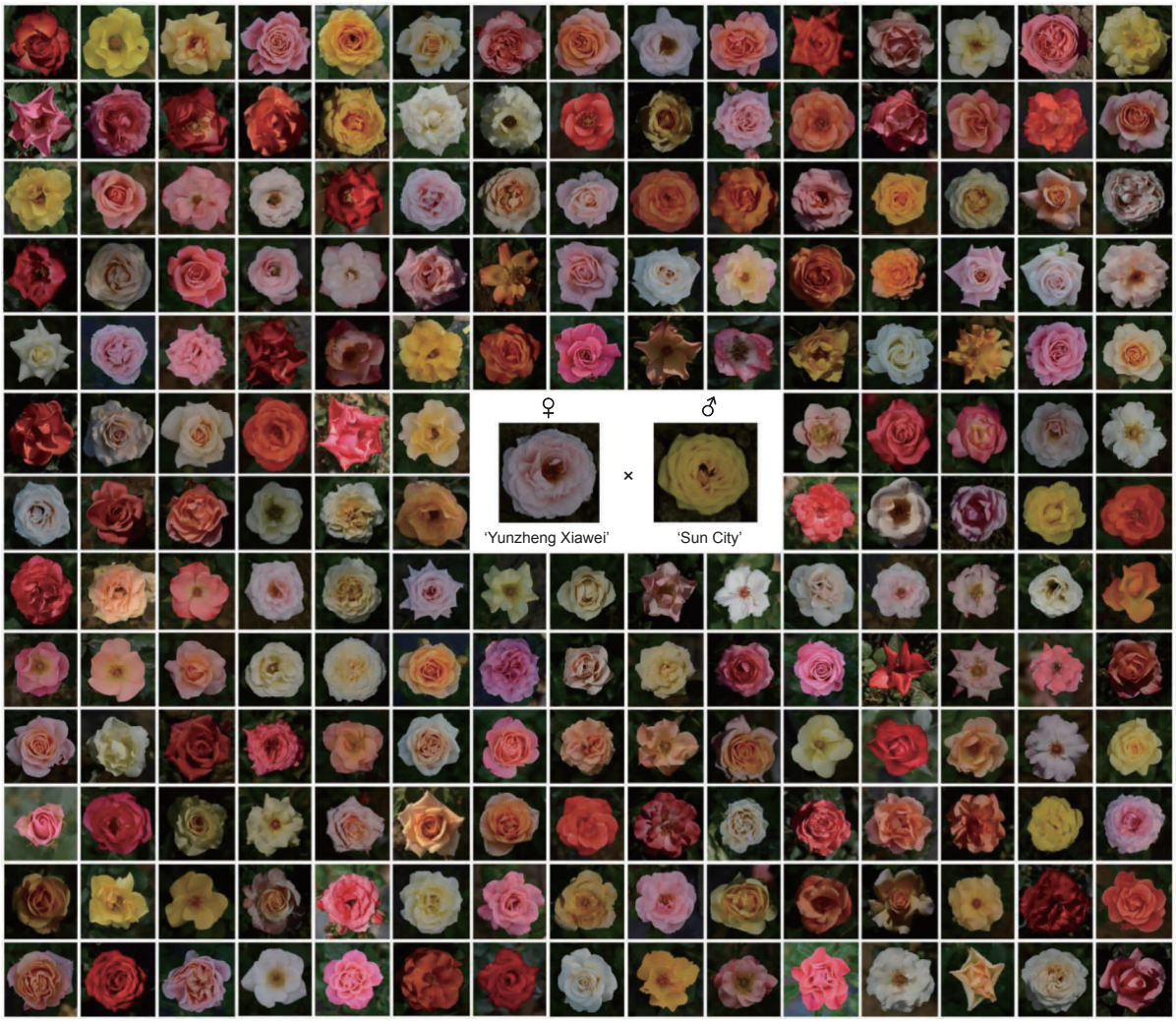

Fig. 1 Plate illustrating the floral traits of the tetraploid rose YS ('Yunzheng Xiawei'×'Sun City') population.

Identity-by-descent (IBD) probabilities for the population were estimated using TetraOrigin (Zheng *et al*. 2016), with the following error prior settings: parental error prior probability epsF=0 (default), offspring error prior probability eps=0.5 (to allow for higher genotyping error rates), and setting bivalent decoding=TRUE (i.e., assuming only bivalent pairing during parental meiosis). Markers were binned within bin-windows of 1 cM. Specifically, each 1 cM window had at most one marker of each marker segregation type, and in cases where multiple markers were available, the markers with the fewest missing values were chosen. The resulting IBD probabilities were imported into R (R Core Team 2016) for use in QTL mapping. A genome-wide QTL scan was performed per trait in a weighted linear regression with the IBD probabilities as weights, as previously described (Hackett *et al*. 2013, 2014; Bourke *et al*. 2018a). At each tested position, a LOD score was estimated using the following formula:

$$\mathrm{LOD}=\frac{N}{2}\log_{10}\left(\frac{RSS_0}{RSS_1}\right)$$

where N is the population size; RSS_0 is the sum of squared residuals from the Null model, and RSS_1 is the sum of squared residuals after fitting. Significance thresholds of LOD values were determined per trait using a genome-wide permutation test with 1 000 permutations and controlling for Type I errors at α=0.05.

The QTL results were visualized in R per trait, with the results of separate analyses over multiple years superimposed on a single plot. To facilitate visualization, the LOD profiles on such multi-plots were re-scaled such that the significance thresholds coincided, i.e., the *y*-axis LOD scale generally only corresponds to one set of results per plot. Markers located at the peak of LOD or closest to both ends of the QTL interval were considered to be closely linked to the QTLs. The naming of QTLs was based on previously published guidelines (Mccouch *et al*. 1997).

3. Results

3.1. Analysis of the sequencing data

As shown in Table 2, a total of 125.92 Gb of raw data containing 631.06 M reads was obtained from the SLAF library construction and Illumina sequencing. The numbers of reads were 17.67 and 18.60 M in the paternal and maternal lines, respectively. The average number of reads among individuals was 3.18 M. The average Q30 ratio of sequencing data was 93.97%, and the average GC content was 40.09%, which indicated that the sequencing data were of sufficient quality. The filtered sequence data were mapped to the *R. chinensis* genome, with an average alignment rate of 82.49%, of which the alignment rates of the male and female parents were 82.17 and 82.90%, respectively. The average alignment rate of the F_1 progeny was 82.50%. The distribution of DNA fragment lengths was approximately normal, with a median value of 350 bp, conforming to our expectations (Fig. 2).

In this study, quality-controlled reads were anchored to the genome of *R. chinensis*, and their coverage on the genome is shown in Table 3 and Appendix A. The proportions of the regions with at least 1 or 5 reads are denotated as 1× or 5× coverage, respectively, to the total length of the reference genome. The proportion of the genome with 1× coverage ranged from 10.35–23.52%, of which the 1× coverage of parents were 23.52 and 23.06%, respectively, and the average 1× coverage of the offspring was 13.91%. The proportion of the genome with 5× coverage ranged from 3.26–11.66% of the total length of the genome, of which the 5× coverage of the parents were 11.66 and 10.88%, respectively, and the average 5× coverage of the offspring was 96%. The average sequence depths of the parents were 6.65× and 7.03×, respectively, and that of the offspring was 1.19×. The distribution of base coverage depth was uniform across the genome of *R. chinensis*, indicating that the sequenced target space was essentially random.

3.2. Development of genotyping methods

The results of the detection of GATK mutations are shown in Table 4. There were 2 308 019 and 2 308 066 SNP markers detected in the paternal and maternal lines, respectively, of which the numbers of transformed SNP markers were 283 107 and 254 401, which were approximately 1.6 times higher than that of the SNP markers with transmutation. The numbers of parental heterozygous loci were 100 916 and 95 479, respectively, and the homozygous loci numbered

Table 2 Statistics of sequencing data for the tetraploid rose YS ('Yunzheng Xiawei'×'Sun City') population

Sample[1)]	Clean bases (bp)	Clean reads	Q30 (%)[2)]	GC (%)	Mapped ratio (%)
SC (♂)	3 530 131 148	17 672 140	93.66	39.42	81.17
YX (♀)	3 716 262 448	18 598 512	93.12	39.42	82.90
Average of F_1	634 606 271	3 180 674	93.98	40.10	82.50
Total	125 917 766 268	631 056 745	93.97	40.09	82.49

[1)] SC, 'Sun City'; YX, 'Yunzheng Xiawei'.
[2)] Percentage of bases with sequencing quality greater than or equal to 30.

2207103 and 2212587, respectively. The average number of SNP markers detected in the offspring was 2308042. The average numbers of SNP markers with transitions and transversions were 172410 and 104488, respectively, and the former was about twice that of the latter.

In this study, SNP markers were filtered according to a minimum read depth of either 10× (sequencing depth in both parents and offspring is greater than 10×) or 20× (sequencing depth in both parents and offspring is greater than 20×). Genotype calling was similarly performed using both GATK and updog software, resulting in four combined approaches. Finally, a collinearity analysis was conducted by comparing the marker positions on the reference genome with those on the genetic maps. The results using genetic maps obtained by these four methods differ substantially (Fig. 3). The collinearity of GATK-10× and GATK-20× was much higher than that of updog-10× and updog-20×. Generally speaking, GATK-10× was found to generate the best collinearity. In the genetic map of autotetraploid rose constructed by GATK-10× markers, the genetic distances of molecular markers on seven LGs corresponded well with the physical distances, indicating that the genetic map constructed in this study had a good collinearity with the genome of *R. chinensis*.

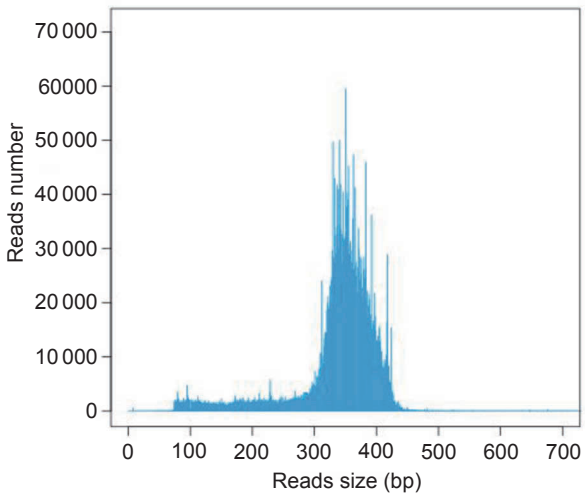

Fig. 2 Distribution of the fragment lengths of sequencing data for the tetraploid rose YS ('Yunzheng Xiawei'×'Sun City') population.

According to the 10× filtration criterion, 17382 molecular markers were screened from the SNP markers that were detected, 440 previously developed SSRs, and AFLP markers, including the 381 SSR and AFLP markers developed in previous studies; and after the removal of non-segregating markers, 6885 high quality markers were finally obtained (Fig. 4). According to the coding rules for autotetraploids, the 6885 screened molecular markers were genotyped using GATK Software, and nine different marker segregation types were identified. Among them, the numbers of single-dose markers were the largest, with 1642 and 1960 markers for 1×0 and 0×1, respectively, comprising 52.32% of all the markers mapped. The number of type 1×1 was 1225 markers, comprising 17.80% of all the markers. The minimum numbers of type 2×2 and type 1×3 were 283 and 225 markers, comprising 11 and 3.27% of all markers, respectively.

3.3. Construction of the genetic map of tetraploid rose

The genetic linkage analysis of the modern rose F_1 mapping population was conducted with polymapR (Bourke *et al*. 2018b) using 6885 markers. Following the filtration criteria, no individual was eliminated. The genetic map of the parents was first constructed with seven LGs, which is consistent with the number of haploid chromosomes in rose. The

Table 3 Degree of coverage and average depth of sequencing data for the tetraploid rose YS ('Yunzheng Xiawei'×'Sun City') population

Sample	Covered bases (bp)	Coverage 1× (%)	Coverage 5× (%)	Average depth (×)
SC (♂)	121243975	23.52	11.66	6.65
YX (♀)	118889673	23.06	10.88	7.03
Average of F_1	71704745	13.91	96	1.19
Maximum of F_1	89635407	17.39	7.18	2.57
Minimum of F_1	53377540	10.35	3.26	0.63

Table 4 Statistical analysis of SNP markers in the tetraploid rose YS ('Yunzheng Xiawei'×'Sun City') population

Sample	SNP	Transition[1)]	Transversion[2)]	Heterozygosity	Homozygosity
YX (♀)	2308019	283107	174368	100916	2207103
SC (♂)	2308066	254401	155014	95479	2212587
Average of F_1	2308042	172410	104488	51184	2256857

[1)] The replacement of a base with the same kind of base (from purine to purine or pyrimidine to pyrimidine).
[2)] The replacement of a base with a different kind of base (from purine to pyrimidine or pyrimidine to purine).

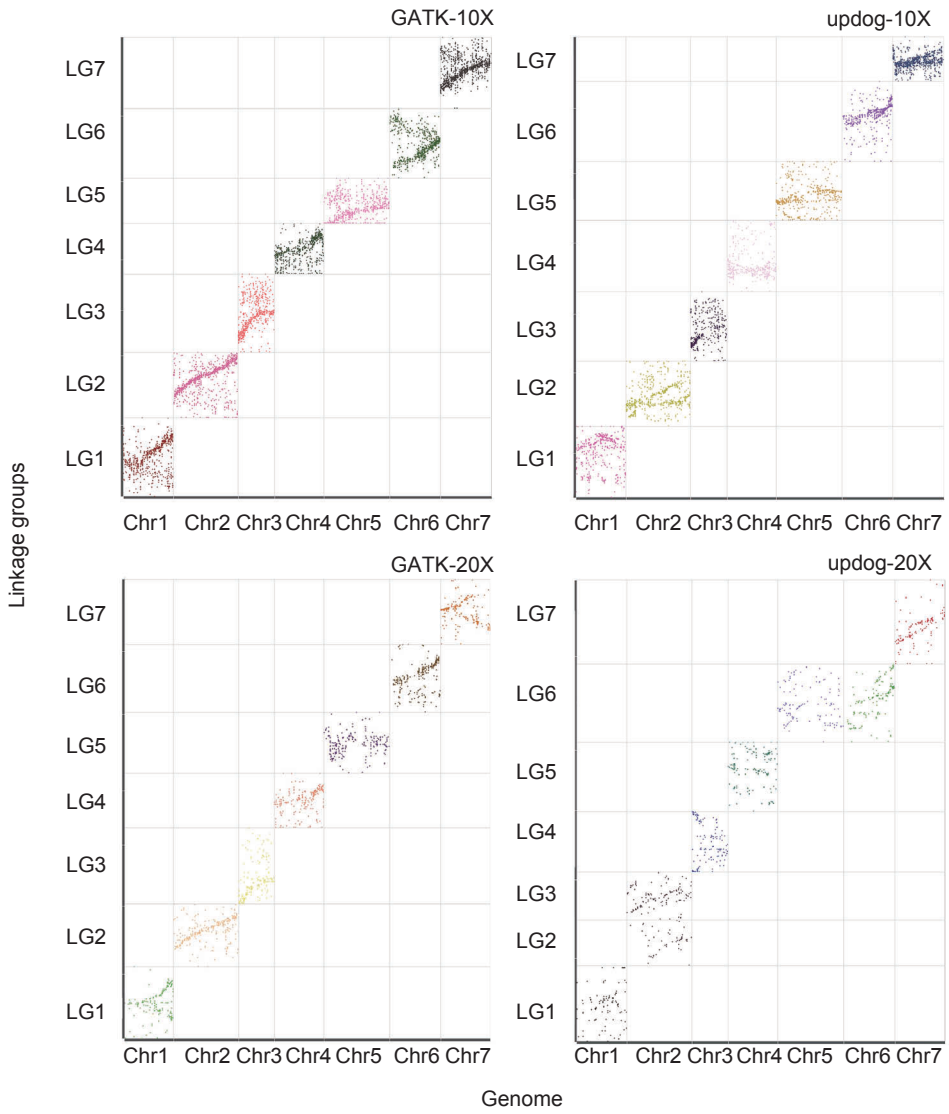

Fig. 3 Results of the collinearity analysis of the tetraploid rose YS ('Yunzheng Xiawei'×'Sun City') population based on four different methods.

paternal genetic map also contained seven LGs with 4 434 markers; a total map length of 1 146.18 cM, and an average map spacing of 0.27 cM, which indicates that the density of the paternal genetic map is high. The length of each linkage group ranged from 127.27 to 187.79 cM, with an average length of 163.74 cM, while the number of markers ranged from 468 to 883, with an average of 633. The maximum distance between markers was 12.75 cM, located near the telomere of LG3. LG3 was also the largest linkage group with 626 markers, and the average distance between markers was 0.30 cM, while LG4 was the smallest linkage group with 492 markers and an average distance of 0.26 cM.

The total length of the maternal genetic map was 1 298.77 cM, longer than that of the paternal genetic map. The number of markers was 4 837, which was greater than that of the paternal genetic map. The length of each LG ranged from 166.77 to 231.46 cM, with an average length of 185.54 cM, while the number of markers ranged from 522 to 1 041 with an average marker number of 691 and an average distance of 0.28 cM, which indicates that the density of the maternal genetic map is higher than that of the paternal genetic map. The maximum distance between markers was 21.60 cM, located near the telomere of LG2. LG2 was the largest linkage group with 883 markers, and the average distance between the markers was 0.26 cM, while LG1 was the smallest linkage group with 574 markers and an average distance of 0.29 cM.

The genetic maps of the parents were further integrated using bi-parental markers, and the results are shown in Table 5 and Fig. 5. The integrated genetic map of the

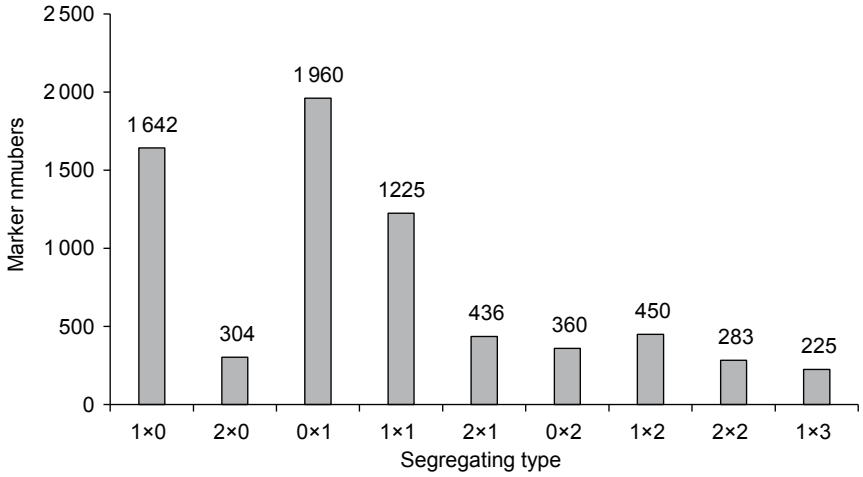

Fig. 4 The number of markers in each marker segregation type on the linkage maps of the tetraploid rose YS ('Yunzheng Xiawei'×'Sun City') population.

Table 5 Markers of maternal and parental linkage groups of the tetraploid rose YS ('Yunzheng Xiawei'×'Sun City') population on the integrated linkage map

Group ID	Total number of markers	Total distance (cM)	Average distance (cM)	Max gap (cM)
LG1	775	199.46	0.26	154
LG2	1 248	1626	0.13	5.52
LG3	827	197.71	0.24	5.83
LG4	764	128.34	0.17	71
LG5	858	113.93	0.13	2.92
LG6	898	175.65	0.20	6.44
LG7	1 472	179.55	0.12	29.16
Total	6 842	1158.90	–	–
Average	977	165.56	0.18	9.88

parents contained seven LGs, with a total map spacing of 1 158.90 cM, 6 842 markers, and an average genetic distance of 0.18 cM between markers, which indicates that the density of the integrated genetic map of the parents is very high. The length of each LG ranged from 113.93 to 199.46 cM, with an average length of 165.56 cM. The number of markers ranged from 764 to 1 472, and the average marker number was 977. The maximum distance between markers was 29.16 cM, located near the telomere of LG7. LG1 was the largest linkage group with 775 markers, and the average distance between the markers was 0.26 cM, while LG5 was the smallest linkage group with 858 markers and an average distance of 0.13 cM.

3.4. Genetic variation of flower-size relevant traits in tetraploid rose

The number of petals and flower diameter in the YS population exhibited bi-directional transgressive segregation. As shown in Fig. 6 and Table 6, the coefficient of variation (CV=Standard deviation/Mean) of the number of petals in the YS population was relatively large, with the maximum CV in 2014 of 72.78%, and the minimum CV of the average number of petals for the three years (2014, 2015, and 2016) was 70.55%. Compared with the number of petals, the CV of flower diameter was relatively small; the maximum CV in 2015 was 19.22%, and the minimum CV of the average flower diameter in 2015 and 2016 was 17.57%. The CV of number of petals and flower diameter in different years was more than 10%, indicating that the genetic variation of the YS population in petal number and flower diameter was large. The ANOVA analysis of the investigated phenotype showed that the petal number and flower diameter did not differ significantly (P<0.05) among the different years (Appendix B). These results indicate that flower-size relevant traits were less affected by environmental factors.

The frequency distributions of petal number and flower diameter in the YS population were analyzed in more detail, and the skewness values of –0.20 to 1.71 indicated that the petal number and flower diameter in different years were normally distributed in the YS population (Table 6).

A correlation analysis of seven measurements (i.e., data

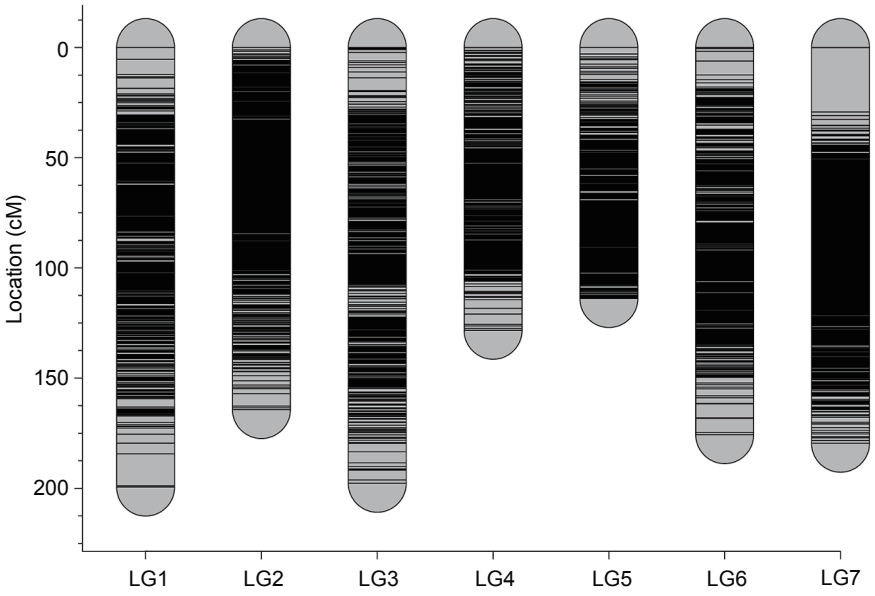

Fig. 5 Integrated linkage map of maternal and paternal linkage groups of the tetraploid rose YS ('Yunzheng Xiawei'×'Sun City') population.

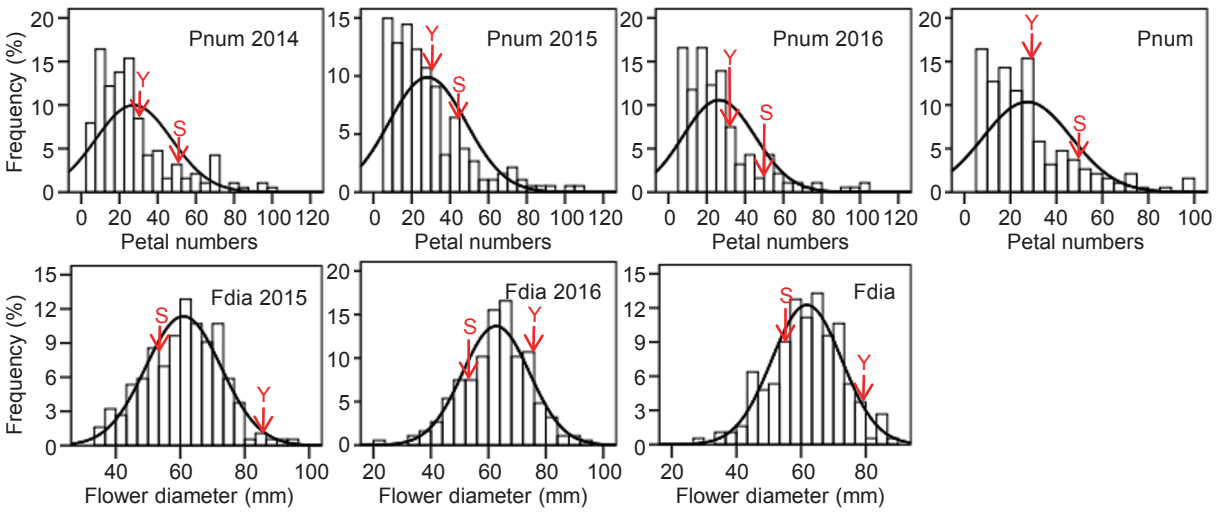

Fig. 6 Frequency distributions of the number of petals and flower diameter in the tetraploid rose YS ('Yunzheng Xiawei'×'Sun City') population. pnum 2014, pnum 2015, and pnum 2016 represent the petal numbers in 2014, 2015, and 2016, respectively; pnum represents the average number of petals in 2014, 2015, and 2016; fdia 2015 and fdia 2016 represent the flower diameters in 2015 and 2016, respectively; fdia represents the average flower diameter in 2015 and 2016.

from the measurements of petal number and flower diameter taken in different years) related to the number of petals and the flower diameter of the YS population in different years is shown in Fig. 7. There was a highly significant positive correlation between the number of petals and flower diameter in different years (P<0.01), indicating that the data were repeatable between different years, and the petal number and flower diameter were primarily controlled by genetic factors but were less affected by environmental factors. The number of petals was significantly positively correlated with flower diameter in different years (P<0.05), which revealed that these traits were closely related.

3.5. QTL analysis of petal number and flower diameter

The results of the QTL analysis of petal number and flower diameter are shown in Fig. 8 and Table 7. One major QTL (qpnum-3-1), located on LG3 was detected for the

Table 6 Statistical analysis of the number of petals and the flower diameter of the tetraploid rose YS ('Yunzheng Xiawei'×'Sun City') population

Trait	Number of individuals	Average	Minimum	Maximum	Skewness	Kurtosis	Coefficient of variation (%)
pnum 2014	187	27.44	5.00	101.00	1.45	1.83	72.78
pnum 2015	185	28.23	5.00	107.67	1.47	2.28	71.52
pnum 2016	185	26.38	5.00	102.67	1.71	3.45	71.52
pnum	187	27.30	5.00	99.78	1.51	2.43	70.55
fdia 2015	185	60.96	34.02	94.63	–0.08	–0.17	19.22
fdia 2016	185	62.67	23.61	94.98	–0.23	0.46	18.63
fdia	186	61.75	28.82	88.51	–0.20	–0.03	17.57

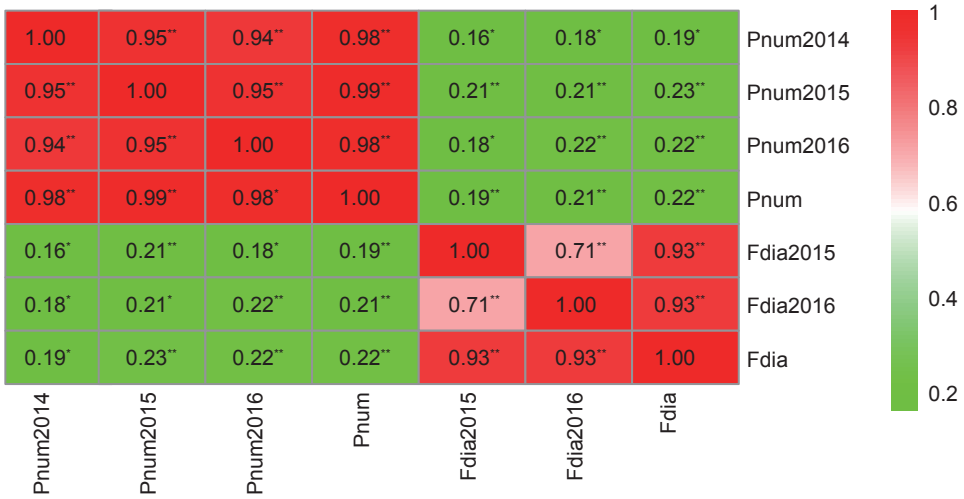

Fig. 7 A heat map of the correlation matrix of the number of petals and flower diameter in the tetraploid rose YS ('Yunzheng Xiawei'×'Sun City') population.

number of petals, and it was detected in three consecutive years and the average of these three years. The marker chr3_9354935 was closely linked to qpnum-3-1 in 2014 and 2015, and the average of the three years. qpnum-3-1 explained 20.18–22.11% of the variation in the number of petals in different years. The SNP was physically located in the intron at 9 354 935 bp on chromosome 3. The putative impact was modified, and the corresponding gene was RchiOBHmChr3g0461291.

Four QTLs were found to control the flower diameter, and they were distributed on LG2, LG4, and LG7. In 2015 and 2016 and the average of the two years, three, two, and three QTLs were detected, respectively. The main locus, qfdia-2-2, was apparent in the two consecutive years and the average of two years, and their closely linked markers were chr2_5380444, chr2_53637477 and chr2_5380444, respectively. qfdia-2-2 explained 11.12–12.56% of the variation in flower diameter in the years measured in this study. The SNP was physically located in the intron at 5 308 444 bp on chromosome 2. The corresponding gene was RchiOBHmChr2g0091711.

4. Discussion

With the development of high-throughput sequencing, its application for the detection and exploitation of genomic polymorphisms has become increasingly widespread (Barabaschi *et al*. 2016; Senthilvel *et al*. 2019). Compared with traditional molecular marker techniques (AFLP, Restriction Fragment Length Polymorphism (RFLP) and SSR), SLAF-seq has the advantages of efficiency and a rapid turnaround (Sun *et al*. 2013; Scheben 2016). SLAF-seq has been widely used in the development of molecular markers and the construction of genetic maps of various species, such as *Prunus mume* (Zhang *et al*. 2015), *Benincasa hispida* (Jiang *et al*. 2015), *Strongylocentrotus intermedius* (Chang *et al*. 2018), *Oryza sativa* (Ying *et al*. 2018), *Vitis* (Lin *et al*. 2019) and *Lycium* (Zhao *et al*. 2019). In addition, it has been applied successfully in many polyploid species. Zhao *et al*. (2016) developed molecular markers for an F_1 population of autotetraploid orchardgrass (*Dactylis glomerata* L.) using SLAF-seq and constructed a genetic map using diploid marker typing

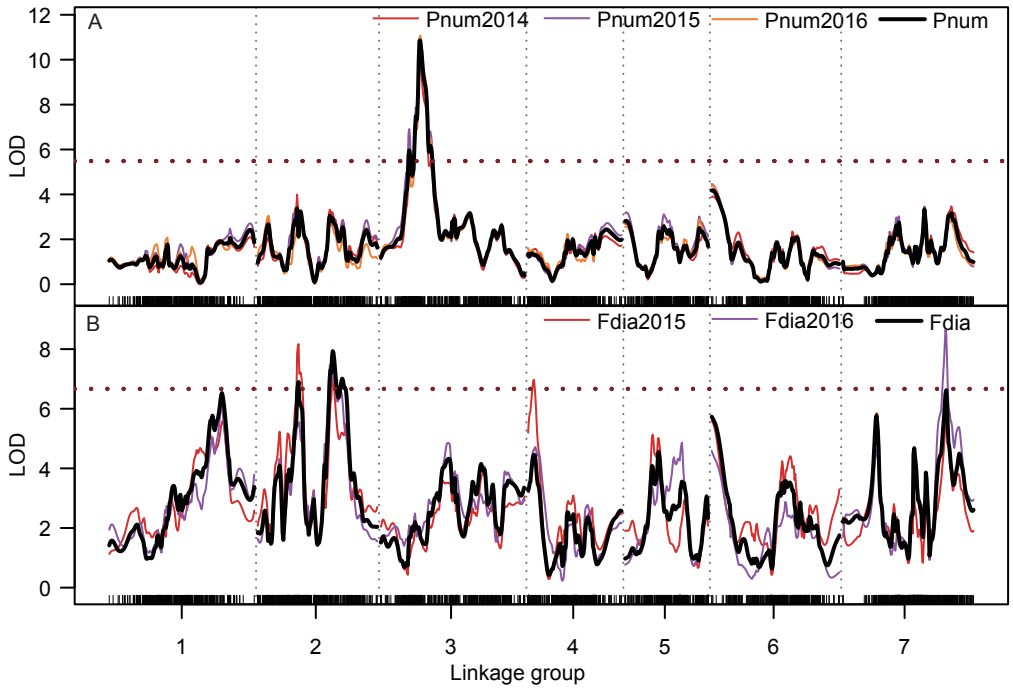

Fig. 8 QTL mapping for the number of petals (A) and flower diameter (B) in different years in the tetraploid rose YS ('Yunzheng Xiawei'×'Sun City') population. LOD, logarithm of odds.

Table 7 Summary of QTLs of petal numbers and flower diameter in different years in the tetraploid rose YS ('Yunzheng Xiawei'×'Sun City') population[1)]

Trait	Year	QTL	LG	Position (cM)	LOD	Marker	Confidence interval (cM)	R^2 (%)
pnum	2014	qpnum-3-1	3	53.16	10.48	chr3_9354935	46.41–64.64	20.18
	2015	qpnum-3-1	3	53.16	11.37	chr3_9354935	37.31–70.18	22.11
	2016	qpnum-3-1	3	54.09	11.21	chr3_8105458	37.75–70.18	21.81
	Average	qpnum-3-1	3	53.16	11.48	chr3_9354935	37.75–70.18	22.11
fdia	2015	qfdia-2-1	2	56.27	7.18	chr2_43944842	53.25–60.46	13.54
	2015	qfdia-2-2	2	103.47	6.07	chr2_5380444	101.92–103.51	11.12
	2015	qfdia-4-1	4	7.7	6.1	chr4_50172471	7.42–8.99	11.18
	2016	qfdia-2-2	2	99.25	6.49	chr2_53637477	97.45–106.05	12.04
	2016	qfdia-7-1	7	141.43	7.54	chr7_3037003	137.04–143.81	14.31
	Average	qfdia-2-1	2	56.39	6.21	chr2_48226057	54.15–56.49	11.37
	Average	qfdia-2-2	2	103.47	6.75	chr2_5380444	99.04–119.26	12.56
	Average	qfdia-7-1	7	141.45	5.66	chr7_5859057	141.43–141.45	10.16

[1)] LG, linkage group; LOD, logarithm of odds

and map construction methods. Zhuang *et al*. (2019) developed two new genetic maps of the RIL populations of peanut (*Arachis hypogaea*) using SLAF-seq. They proceeded to integrate them with two previous linkage maps to support chromosome assembly and perform a QTL analysis. However, new methods and software are becoming increasingly available for genetic mapping and QTL analysis in polyploids, allowing for improved genetic analyses of polyploid species (Bourke *et al*. 2018c). To the best of our knowledge, polyploid-specific software, such as polymapR (Bourke *et al*. 2018b) or TetraploidSNPMap (Hackett *et al*. 2017), has only been applied in studies where genotyping was performed using SNP arrays, for which they were originally developed (Bourke *et al*. 2017). In this study, the genetic loci of tetraploid roses were obtained using simplified genome sequencing (SLAF-seq), followed by map construction and QTL analysis. Therefore, this study also provides a good proof-of-principal for the genetic analysis of other autopolyploid plants using high-throughput sequencing for genotype assignment.

The density of the genetic map of tetraploid rose constructed in this study was greatly improved compared

with traditional molecular marker technologies. In our study, the integrated parental linkage map contained 6 842 markers, and the average genetic distance between markers was 0.18 cM. In a previous study on the YS population, Yu *et al*. (2014) used SSRs and AFLPs to construct a linkage map with 295 markers and an average genetic distance of 2.9 cM. Bourke *et al*. (2017) constructed an ultra-high-density linkage map with 25 695 SNP markers, and genotyping was conducted using the WagRhSNP 68K array (Koning-Boucoiran *et al*. 2015). Compared with those obtained in our study, the genetic maps created by Bourke *et al*. (2017) possessed more markers, which may be due to the fact that SNPs on the array (WagRhSNP 68K array) were identified to a large part using the same K5 mapping population. It is also the case that the stringent data filtering steps used in this study removed a large number of markers, which is one of the drawbacks of using an error-prone genotyping methodology. That said, within the constraints of a bi-parental mapping population, only a limited number of recombination events are available to guide marker ordering. With almost 7 000 mapped markers, our genotyping method provided more than ample genetic resolution and would be a suitable choice for other autopolyploid species.

In this study, qpnum-3-1 was found to be the major QTL that controls the number of petals. This is consistent with the results of the diploid integration map obtained by Spiller *et al*. (2011), which are closely linked to Rw11E5-181 markers (Appendix C). It is highly likely that the major QTL for petal number identified on LG3 also corresponds to the *DOUBLE FLOWER* locus on chromosome 3, for which a candidate *APETELA2/TOE* gene was recently proposed (Hibrand Saint-Oyant *et al*. 2018). Interestingly, petal number was positively correlated with the flower diameter in the YS population, but no common QTL was detected. This is a somewhat unexpected result, although a QTL for petal number was previously identified on LG2 (Bourke *et al*. 2018a), which may coincide with the flower diameter QTL detected here. Alternatively, this discrepancy could be explained by other independent factors that can contribute to flower diameter, such as petal elongation or petal arrangement. The QTL that controls petal number was consistently detected in different years, indicating that qpnum-3-1 was less affected by environmental factors and could be a stable marker for use in the marker-assisted breeding of rose.

5. Conclusion

In this study, a high-density linkage map of rose was constructed by restriction-site associated DNA sequencing and genetic software developed for autopolyploids. The QTLs for the number of petals and flower diameter were then analyzed. A high-density genetic linkage map of tetraploid rose was constructed by polymapR using 6 885 markers, with 6 842 markers distributed on seven linkage groups. The map spanned 1 158.90 cM with an average inter-marker distance of 0.18 cM. qpnum-3-1 was found to be the major QTL that controlled the number of petals, and qfdia-2-2 was found to be the major QTL that controlled flower diameter. Our findings suggest that untargeted sequence-based genotypes can be deployed successfully in autopolyploid species, demonstrating a viable alternative to more traditional targeted genotyping approaches which have formed the bulk of polyploid gene forward genetic studies to date.

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Declaration of competing interest

The authors declare that they have no conflict of interest.

Appendices associated with this paper are available on http://www.ChinaAgriSci.com/V2/En/appendix.htm

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