An ultra-high-density genetic map provides insights into genome synteny, recombination landscape and taproot skin colour in radish (Raphanus sativus L.)

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Summary
High-density genetic map is a valuable tool for exploring novel genomic information, quantitative trait locus (QTL) mapping and gene discovery of economically agronomic traits in plant species. However, high-resolution genetic map applied to tag QTLs associated with important traits and to investigate genomic features underlying recombination landscape in radish (Raphanus sativus) remains largely unexplored. In this study, an ultra-high-density genetic map with 378 738 SNPs covering 1306.8 cM in nine radish linkage groups (LGs) was developed by a whole-genome sequencing-based approach. A total of 18 QTLs for 11 horticulture traits were detected. The map-based cloning data indicated that the R2R3-MYB transcription factor RsMYB90 was a crucial candidate gene determining the taproot skin colour. Comparative genomics analysis among radish, Brassica rapa and B. oleracea genome revealed several genomic rearrangements existed in the radish genome. The highly uneven distribution of recombination was observed across the nine radish chromosomes. Totally, 504 recombination hot regions (RHRs) were enriched near gene promoters and terminators. The recombination rate in RHRs was positively correlated with the density of SNPs and gene, and GC content, respectively. Functional annotation indicated that genes within RHRs were mainly involved in metabolic process and binding. Three QTLs for three traits were found in the RHR. The results provide novel insights into the radish genome evolution and recombination landscape, and facilitate the development of effective strategies for molecular breeding by targeting and dissecting important traits in radish.

Introduction
High-density genetic maps are crucial for genetic and genomic studies, such as mapping quantitative trait loci (QTLs) associated with important agronomic traits in crops. QTL mapping resolution is dependent on marker density and genetic population size (Xu et al., 2013). High-throughput resequencing has been proposed to facilitate the development of markers, genotyping, and increase the marker density of genetic maps (Cao et al., 2011; Hu et al., 2017). A sliding window approach combining with whole-genome resequencing (WGRS) data was employed for genotype calling and recombination breakpoint determination (Huang et al., 2009; Xu et al., 2013). It has been widely applied to construct the recombiant bin map based on the SNP markers and identify important QTLs or genes in various important crops including rice (Gao et al., 2013), Brassica rapa (Yu et al., 2013) and soybean (Lu et al., 2017). A bin map was constructed by genotyping a recombinant inbred line (RIL) population and identified the candidate genes involved in yield-associated trait in rice (Gao et al., 2013). In soybean, the high-density maps generated from WGRS were also effectively applied in mapping a QTL harbouring candidate genes PP2C for seed weight (Lu et al., 2017). Using high-density map based on sequencing-based genotyping method, these findings provide solid basis for QTL mapping associates with important traits.

Anthocyanins, an important flavonoid compound, are responsible for the different colours of varying vegetables, flowers and fruits (Jaakola, 2013; Yuan et al., 2009, 2016). Anthocyanins possess potent antioxidant capacities, providing a variety of health-promoting benefits including protection against cardiovascular disease, obesity and diabetes (He and Giusti, 2010; Nabavi et al., 2015). Increasing number of studies showed that anthocyanins play important roles in attracting pollinators and seed dispersers, as well as protecting high light stress and pathogen attack (Allan et al., 2008; Grotewold, 2006; Yamagishi et al., 2014). The biosynthesis of anthocyanin is mainly regulated by the expression of structural genes at the transcriptional level in plants. In apple, the MdMYB10 alleles were shown to be correlated with anthocyanin accumulation and were higher in red-fruited than in green-fruited cultivars (Ban et al., 2007; Takos et al., 2006). The PwMYB10.1 alleles regulate anthocyanin accumulation in sweet cherry skin (Jin et al., 2016). It is well known that MYB, basic helix–loop–helix (bHLH) transcription factors (TFs) and a WD-repeat (WDR) protein (MBW complex) can coordinately activate the late steps towards anthocyanin and proanthocyanidin pathway, which binds the promoters of structural genes to induce their transcription (Koes et al., 2005; Xu et al., 2015b). The QTL underlying red pigmentation of radish taproot was mapped on LG11 at positions 29.1 and 56.1 cM, respectively (Tsuru et al., 2013).
Meiotic recombination is an essential biological process for the generation of new combinations of alleles and plays an important role in genomic evolution (Si et al., 2015). Recombination contributes to influence the plant breeding programmes by affecting the efficiency of natural selection (Silva-Junior and Grattagpaglia, 2015). High-density genetic map, as a classic tool to study recombination, can be measured through comparing genetic and physical distances (cM/Mb) on the genome. The distribution of recombination is not uniformly distributed along the genome, and the location of recombination is more likely to occur in gene transcriptional start sites (TSSs) and transcriptional termination sites (TTTs) (Choi et al., 2013; Pan et al., 2016). The numerous genomic features can directly influence the recombination rates, such as GC content, DNA polymorphism, density of gene and transposable elements (Auton et al., 2012; Hunter et al., 2016). The recombination events occur more frequently than in other regions were defined as recombination hotspots. It was shown that the CpCYC-B gene has been identified to be associated with fruit flesh colour and located in the recombination hotspot region (Blas et al., 2010). The intraspecific variation for recombination was observed in different maize populations, and intragenic recombination significantly correlates with gene expression and phenotypic variation by construction of high-density genetic map, suggesting the potential roles for recombination in plant phenotypic variation (Bauer et al., 2013; Pan et al., 2016). To date, little information is known about the genome-wide recombination and genomic feature correlation with recombination rate in radish.

Radish (Raphanus sativus L., 2n = 2x = 18) is an important root vegetable crop of Brassicaceae family. Comprehensive identification of key genes associated with root-related traits is the prerequisite for exploitation in radish genetic improvement. In the past decade, several radish genetic maps had been constructed by resequencing a F 2 population derived from a cross between ‘NAU-LB’ and ‘NAU-YH’. A large number of QTLs were identified for some important traits basing on the high-density genetic linkage map. The candidate gene associated with taproot skin colour was isolated using QTL mapping and map-based cloning techniques. Furthermore, the high-density genetic map was used to perform comparative genomics analysis and explore the genome-wide patterns of recombination. These results could provide valuable resource for enhancing our understanding of genome evolution and recombination landscape, and facilitate effective fine mapping and isolating of quantitative trait genes controlling important traits in radish.

**Results**

**Population sequencing and linkage map construction in radish**

To construct a high-resolution genetic linkage map in radish, the whole-genome resequencing of 137 F 2 individuals together with the parental lines was performed on Illumina HiSeq™ 2500 platform. In total, 7.4 Gb (17.3-fold genome coverage) and 5.6 Gb (12.7-fold genome coverage) clean reads were generated for the ‘NAU-LB’ and ‘NAU-YH’, respectively (Table S1). A total of 403 Gb clean reads were generated for the 137 F 2 individuals, with an average of 7.2 depth for each individual. The clean reads were mapped against the newly available radish reference genome. A total of homozygous 821 217 SNPs were detected between the two parental lines. After filtering SNPs by the genotyping criteria, 411 891 SNPs were retained to generate bin markers among F 2 population. The SNP with significant segregation distortion (P < 0.001) was excluded for further analysis. A modified sliding window approach was performed to determine recombinant breakpoints for the F 2 individuals. The adjacent bins with the same genotype were merged as a bin, and a high-density genetic map was constructed with 2852 recombination bin markers (378 738 SNPs) (Figures 1 and S1). The total genetic distance of the bin map was 1306.8 cM, with an average distance of 0.46 cM between adjacent bin markers (Tables 1 and S2). These bin markers ranged from 85.73 to 231.51 kb, with an average physical length of 120.91 kb. According to the bin marker locations on the radish reference genome, a total of 40 802 genes were found in 2852 bins, ranging from 3285 to 6317 genes in each chromosome.

In total, 335 (11.75%) and 270 (9.47%) bin markers exhibited significant segregation distortion at the P < 0.005 and P < 0.001, respectively (Figure S2, Table S3). Among the distorted markers, 316 (93%) bins skewed to the paternal alleles (‘NAU-YH’), while 11 (3.28%) bins skewed to the maternal alleles (‘NAU-LB’) and 8 (2.39%) segregated in favour of the heterozygous alleles. A distorted region with more than three adjacent loci was considered as a segregation distortion region (SDR). A total of 19 SDRs were identified on seven linkage groups (LGs) with LG6 (7 SDRs) having the largest number of SDRs (Table S4). Since the segregation distortion has less effect on mapping accuracy and could increase the coverage of the linkage groups (Bartholomé et al., 2015), the distorted bin markers were retained in the linkage maps.

**QTL mapping of important horticulture traits in radish**

Phenotypic trait data in F 2 populations exhibited approximately normal distributions (Figure S3). The negative and positive correlations between each pair among the ten traits were evaluated (Table S5). A total of 17 QTLs were detected for ten traits in the bin map using MQM analysis, seven of which were mapped onto the same LG with the reported QTL, providing support for the accuracy of this map (Yu et al., 2016) (Table S6). All identified QTLs with 8.1–21.2% phenotypic variance were mapped to five LGs. The regions of identified QTLs ranged from 51 to 527 kb, with an average length of 168 kb.

To isolate the taproot skin colour gene, the ‘NAU-LB’ (white skin colour) was crossed with ‘NAU-YH’ (red skin colour) (Figure 2a). The anthocyanin content of ‘NAU-YH’ was higher than ‘NAU-LB’ (Figure 2b). All F 1 plants exhibited red root skin, and the F 2 individuals were classified as red and white. The
segregation of F2 generation fitted a 3:1 ratio in two consecutive years ($\chi^2 = 0.002$ in 2016 and $\chi^2 = 0.896$ in 2017). Trait segregation in BC1P1 populations was the ratio of 1:1 ($\chi^2 = 0.06$; Table 2). The taproot skin of all the BC1P2 populations was red. This genetic analysis confirmed that the root skin colour is controlled by a dominant gene, which was designated as $R$. To identify the causal gene underlying the taproot skin colour, clean reads from 10 red skin plants and 10 white skin plants were collected. A total of 187 million and 191 million clean reads were generated for the R-bulk (47.26-fold genome coverage) and W-bulk (48.3-fold genome coverage), respectively (Table S7). After eliminating the low-quality SNP index in two bulks, 2 500 223 SNPs were identified on all nine chromosomes. A $D_{SNP}$ (SNP index) graph was calculated and plotted against the genome positions, and the $R$ locus was located to 2.6-Mb (9.0–11.6 Mb) interval on chromosome 7 (Figure 2). A total of 719 938 InDels were identified between R-bulk and W-bulk, 34 of the InDels in candidate interval were selected to amplify polymorphisms among the parental lines and two DNA bulks. Ten polymorphic markers were used to screen the F2 individuals, and the $R$ locus was anchored between the InDels markers RsInDel4 and RsInDel11. The $R$ gene was narrowed down to a 74-kb genomic region between the InDels markers RsInDel8 and RsInDel11 using 145 F2 plants with the white skin phenotype (Figure 2e).

Quantitative trait locus analysis for taproot skin colour was performed using MapQTL based on the high-density genetic map. A single locus (bin marker 2194) located on LG 7 was the most significant markers according to the Kruskal–Wallis test ($K = 105.83$, $P < 0.0001$; Figure 2d). The peak LOD in LG 7 was also located at bin 2194, with highest LOD values of 45.24 by interval mapping (Figure S4). Finally, the $R$ locus was mapped to a 72-kb region, indicating the high accuracy of high-density genetic map in this study. Thirteen putative open reading frames (ORFs) were predicted based on the radish reference genome information (Table S8). An Rs388430 was predicted to belong to the R2R3 MYB transcription factor family, which was a homologous of AtMYB90/PAP2 involved in regulation of the anthocyanin biosynthetic pathway (Gonzalez et al., 2008). RT-PCR and RT-qPCR analysis revealed that the expression level of RsMYB90 was higher in taproot skin of ’NAU-YH’ than that in ’NAU-LB’ (Figure S5a,b), indicating RsMYB90 was the candidate gene for the $R$ controlling the red skin colour.

Table 1 Summary of the nine linkage groups in radish

| Linkage group | Number of SNP | Number of bin | Genetic distance (cM) | Average distance (cM) | Average length of bin (kb) | Number of gene | Recombination rate (cM/Mb) |
|---------------|---------------|---------------|-----------------------|-----------------------|---------------------------|----------------|---------------------------|
| LG01          | 28 947        | 217           | 128.16                | 0.59                  | 128.16                    | 3285           | 4.87                      |
| LG02          | 57 202        | 372           | 85.73                 | 0.23                  | 85.73                     | 5229           | 1.96                      |
| LG03          | 43 429        | 231           | 98.37                 | 0.43                  | 98.37                     | 3345           | 3.38                      |
| LG04          | 38 657        | 400           | 190.06                | 0.48                  | 190.06                    | 5992           | 3.80                      |
| LG05          | 61 262        | 374           | 163.63                | 0.44                  | 163.63                    | 5886           | 3.56                      |
| LG06          | 67 894        | 453           | 231.51                | 0.51                  | 231.51                    | 6317           | 4.32                      |
| LG07          | 25 133        | 233           | 103.58                | 0.44                  | 103.58                    | 3293           | 3.81                      |
| LG08          | 21 230        | 235           | 139.78                | 0.59                  | 139.78                    | 3302           | 4.71                      |
| LG09          | 34 984        | 337           | 165.98                | 0.49                  | 165.98                    | 4153           | 4.33                      |
| Total         | 378 738       | 2852          | 1306.80               | 0.46                  | 120.91                    | 40802          | 3.80                      |

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Figure 1 Recombination bin map (a) and genetic map (b) of 137 F2 individuals.

Figure 2 a) Recombination bin map and b) genetic map of 137 F2 individuals.
skin colour of taproot. A total of 16 radish genotypes with different skin colours were employed to investigate the expression profiles of \(RsMYB90\) gene by RT-PCR approach. As shown in Figure S5c, the \(RsMYB90\) gene is highly expressed in eight red skin varieties, whereas it is barely expressed in eight white skin varieties. Phylogenetic analysis of the deduced amino acid sequences showed that \(RsMYB90\) was most closely related to \(Arabidopsis\) PAP2, \(MYB90\), \(MYB113\) and clustered with the several other anthocyanin-related MYB TFs (Figure S6). The alignment of the amino acid sequences revealed that the R2R3 motifs were conserved at the N-terminus and the \(RsMYB90\) has predicted interaction domains for bHLH partners, and a C-terminal consensus sequence, indicating its potential roles in activation of anthocyanin biosynthesis (Figure S7).

Comparison of the linkage map with the radish genome

The locations of bin markers on the genetic map were compared with their physical positions in the radish genome assembly. All the bin markers were mapped on the nine pseudo-chromosomes, representing 80.7% of 424 Mb for the radish reference genome (Figure 3). A high degree of collinearity was observed between the genetic map and the corresponding chromosome. However, there were still few regions displayed inconsistence on several chromosomes. The bins located at 26–43 Mb on chromosome 2 were inverted in upper region of the corresponding LGs. The order of bins on the distal ends of the chromosomes 1, 3 and 4 was inconsistent with the genetic map.

Colinearity of the radish genetic map with \(Brassica\) genome

To gain insight into the evolutionary history of the radish genome, the location of bin markers was searched against the physical map of \(B. rapa\) and \(B. oleracea\) genomes. Among the 2852 bin markers, 2411 (84.5%) and 2385 (83.6%) markers were aligned to \(B. rapa\) and \(B. oleracea\) chromosomes, respectively. Synteny analysis revealed that the radish LG 8 and LG 9 showed higher
conservation with *B. rapa* chromosome 8 and *B. oleracea* chromosome 9, respectively (Figure 4). In contrast, no one-to-one chromosome relationship was presented between the remaining radish linkage groups and the two *Brassica* species. Despite the radish shared the same chromosome number with *B. oleracea*, LG 7 of radish was matched to chromosomes 2, 3, 6 and 9 of *B. oleracea*, indicating that chromosomal rearrangements have occurred between radish and the two *Brassica* genomes after their divergence from the common ancestor.

**Recombination landscape in radish**

To provide a comprehensive overview of recombination in radish, the recombination rate along each chromosome was estimated by comparing genetic and physical distance. The average recombination rate across the entire genome was 3.8 cM/Mb. The high recombination in telomere regions of all nine chromosomes was observed, whereas the recombination was suppressed in the centromere regions (Figures 5 and S8). The number of recombination per chromosome was not significantly positively correlated with the physical length of the chromosomes ($r = -0.02$, $P = 0.9573$, Figure S9). To detect recombination hotspots in the genome, the chromosomal regions with recombination rate greater than 1.0 cM/Mb were defined as recombination hot regions (RHRs). A total of 504 RHRs were identified and unevenly distributed on the nine LGs (Table S9). The length of RHR ranged from 6.1 to 850 kb with one hotspot detected on average every 215 kb. Chromosome 5 had the maximum RHRs, while chromosomes 2 and 3 had the fewest. The highest recombination rate in RHRs (39.97 cM/Mb) was observed on the chromosome 6 (Chr 6: 17 049 937–17 063 645), which was 10.5-fold higher than the genome average recombination rate. Previous studies indicated that recombination hotspot was associated with GC content, nucleotide diversity and gene density (Silva-Junior and Grattapaglia, 2015). The RHRs with high recombination rates were positively correlated with SNP density, gene density and GC content, respectively, which was predominately located in chromosome ends (Figure 5a). Correlations between GC content and recombination rate are consistent with the result that the increased recombination rates drive increasing GC content by the process of GC-biased gene conversion (gBGC) (Singhal et al., 2015).

To investigate the localization of RHRs in the genome, the overlap between RHRs and exons, introns, 5' UTRs, 3' UTRs, 1 kb upstream of TSS (transcription start sites) and 1 kb downstream of TTS (transcription termination sites) was tested. In comparison with random regions, a higher proportion of overlap with exons, introns, 5' UTRs, 3' UTRs, 1 kb TSS and 1 kb TTS was observed in RHRs. The RHRs overlapped exon (14.6%) were higher than...
introns (9.3%), and recombination rates were higher in first exon than in last intron for genes that have \( \geq 5 \) exons (Figure 5b,c). A higher proportion of overlap was enriched near 1 kb TSS (12.3%) and 1 kb (12.2%) TTS, which could be presumed that TSSs and TTSs facilitated RNA polymerase II (Pol II) transcriptional regulation (Choi et al., 2013). Assessments on the average fine-scale recombination rate profiles relative to all genes indicated that the average recombination rate was increased near TSSs and TTSs, and decreased within the transcribed region, in agreement with the random regions (Figure 5d). Gene Ontogeny (GO) analysis showed that the predominant GO terms enriched in RHRs were related to metabolic process and organic substance metabolic process in biological processes (Figure S10). In the cellular component category, binding and catalytic activity represented the most abundant significantly enriched subcategory, demonstrating genes within RHRs may play an important role in metabolic process.

To explore the recombination under genetic control, the detected QTLs were searched against the RHRs. As expected, three QTLs for three traits were located in RHRs. A major QTL originated from recombination \( (qRL8) \) with 8.3% of total phenotypic variance was mapped to bin 2355 of chromosome 8, with a physical length of 114 kb. Twenty-three genes were identified in candidate region, one of which \( (Rs407980) \) encoding a sugar transporter 2 was homologous with \( A t S T P 2 \). Previously studies showed that sugar transport proteins played important roles in sugar acquisition by roots in \( Arabidopsis \) (Chen et al., 2015). It was indicated that the \( Rs407980 \) was involved in sugar

![Figure 4](https://via.placeholder.com/150)

**Figure 4** Pairwise dot-plot comparison of the syntenic relationships between linkage map and *Brassica oleracea* (a) and *B. rapa* (c) pseudomolecules. Circos representation of the genome synteny between radish and *B. oleracea* (b) and *B. rapa* (d).
transport system and acted a candidate gene for root length in radish. Taken together, it could be inferred that the recombination plays vital role in phenotypic variation of radish.

**Discussion**

A high-density genetic map can be efficiently used to identify candidate causal genes for important agronomic traits and comparative genomics, and support the assembly of reference genome. Radish is an important root vegetable crop worldwide, and the insufficiency of molecular markers hindered the effective application of linkage map for MAS of horticulture traits in breeding programmes. In this study, a high-density bin map composed of 2852 bins was constructed and applied to fine mapping of a candidate gene for red skin colour. Comparative genomic analysis provided insights into the evolutionary history of the radish and Brassica genome. This study represents a first report on the comprehensive characterization of recombination landscape in radish. These results provided valuable information on the understanding of genome features and the practice of molecular breeding in radish.

**High-density linkage map for breeding programme and genome assembly**

The sufficiency of molecular markers, population size and the highly efficient genotyping approaches could facilitate improving the resolution of QTL mapping and ultimately map-based cloning. The WGRS and subsequent analyses provide an effective way to develop a high density genetic map (Gao et al., 2013; Hu et al., 2017). In this study, a high-density genetic map with 2852 recombinant bins was constructed and covered 1306.8 cM with an average marker interval of 0.46 cM with an average marker interval of 0.46 cM. Previously, an integrated linkage map using 221 SNPs and 1514 traditional molecular markers was constructed (Kitashiba et al., 2014). Mun et al. (2015) reported a genetic map with 2532 SNPs and 146 PCR-based markers developed from resequencing. However, the marker density of these maps is still limited to pinpoint the genes underlying a considerable number of QTLs. The marker density in

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**Figure 5** The fine-scale profile of recombination hot regions (RHRs) around the genome. (a) Genomic features of RHRs: Genetic positions of the RHRs in the nine radish pseudo-chromosome (ii), count of recombination rates (iii), SNP density (iv), gene density (v) and GC content (vi) within RHRs. (b) Overlap analysis of RHRs with various genomic features (blue) and random regions (green). (c) Recombination rate within exons and introns for genes. (d) Average recombination rate as a function of distance to nearest transcription start site (TSS) and transcription start site (TSS) for all genes in RHRs (blue) and random regions (green).
the present linkage map is significantly higher than that of many reported radish maps (Kitashiba et al., 2014; Mun et al., 2015; Xu et al., 2012). A total of 18 QTLs controlling 11 traits were identified with this high-density map, and the QTLs for the content of soluble solid, soluble sugar and soluble protein were firstly reported in radish. The QTL intervals detected in this study were significantly shorter than previously reported, indicating the improved resolution of the linkage map (Yu et al., 2016).

The red skin with high concentration of anthocyanin is an important agronomic trait in modern radish breeding, but the underlying molecular bases have not been fully disected. High-density genetic map and whole-genome resequencing-based BSA are two effective methods to identify genetic loci associated with a specific trait (Takagi, 2013; Xu et al., 2013). A PyMYB114 gene on LG3 associated with red skin was identified using a high-density genetic map in pear (Yao et al., 2017). The virescent-1 gene has been rapidly and reliably characterized using BSA combined with whole-genome sequencing in cotton (Zhu et al., 2017). Here, the RsMYB90 gene belonging to the R2R3 family was identified by combining BSA with whole genome resequencing and map-based cloning. QTL mapping for red skin further confirmed the reliability of the high-density bin map in this study. It is known that R2R3 MYB transcription factors play essential roles in regulation of anthocyanin biosynthesis in many plants. In apple, MYB1 and MYBA genes mapped to the same locus are responsible for skin colour (Ban et al., 2007; Takos et al., 2006). Previous studies have suggested that three PavMYB10.1 alleles controlled the fruit skin colour in sweet cherry (Jin et al., 2016). Phylogenetic analysis showed that the RsMYB90 gene was highly homologous with Atpap2. Previous studies revealed that the MYB90/PPAP2 gene was involved in anthocyanin accumulation in several plant species such as Arabidopsis and Brassica napus (Ilk et al., 2015; Fu et al., 2017). In Arabidopsis, the gene expression of AtMYB90/PPAP2 was down-regulated with obvious anthocyanin deficiencies by RNAi (Gonzalez et al., 2008). Overexpression of OvPAP2 gene in B. napus produced red anthers and petals, illustrating the feasibility of producing red-flowered oilseed rape (Fu et al., 2017). In this study, co-segregation of RsMYB90 transcript with the skin color indicated the RsMYB90 gene might be responsible for the skin colour trait, and play an important role in anthocyanin accumulation in radish. Sequence analysis indicated that three SNPs were found in the coding region of RsMYB90 gene between the P1 (white skin radish) and P2 (red skin radish) line, which resulted in three amino acid changes at the position of 55, 96 and 216 amino acids in proteins between parental lines (Figure S11a). Furthermore, a total of three InDels and SNPs were identified in the promoter regions of the two parental lines (Figure S11b). Previous studies indicated that the InDel and SNP markers in the promoter sequences play important roles in plant phenotypic determination (Ye et al., 2017; Zhang et al., 2018). It could be speculated that these InDels and SNPs in the coding and promoter regions of two parental lines might lead into differential expression of RsMYB90 gene and affect anthocyanin biosynthesis. Undoubtedly, further functional characterization of these InDels and SNPs would contribute to clarify the molecular regulatory mechanism underlying anthocyanin synthesis in radish. The MYB-bHLH-WD40 (MBW) complexes that regulated the genes involved in the late steps of anthocyanin and proanthocyanin biosynthesis, which have been characterized in many plant species, including A. thaliana (Gonzalez et al., 2008), apple (Xie et al., 2012), maize (Kong et al., 2012) and pear (Yao et al., 2017). In this study, the RsMYB90 has conserved domain to interact with bHLH, indicating that RsMYB90 plays a protective role in control of root skin anthocyanin biosynthesis in radish. These results showed that our high-density genetic map was effective for genetic mapping of important agronomic traits and available to marker-assisted selection in radish breeding.

Alignment of present genetic map to the radish ‘WK10039’ genome revealed a remarkable collinearity, indicating the robust assembly of reference genome. The relative order of markers in some regions showed nonlinearly between the LGs and the corresponding chromosomes, indicating the intrachromosomal rearrangements and genome reshuffling events have occurred between the two lineages (Du et al., 2016). High-quality genetic map played a vital role in anchoring scaffold sequences into pseudo-chromosomes (Li et al., 2017). In radish, 116 Mb (21.8%) of the cv. ‘Aokubi’ scaffolds was anchored to linkage group (Kitashiba et al., 2014), and the other two genomes lacking genetic maps are not anchored into pseudo-chromosomes (Mitsui et al., 2015; Moghe et al., 2014). In the present study, the bin markers in genetic map captured 344 Mb (80.7%) of the ‘WK10039’ genome sequence. Therefore, the high-density linkage map constructed in this study would be useful for QTL mapping and provide a valuable resource to further improve the genome assembly in radish.

Comparative analysis of the homoeologous relationships between radish and two Brassica genomes

The high-density genetic map provided an opportunity to perform a comparison of collinearity between closely related species. Phylogenetic analysis revealed that the Raphanus was phylogenetically closest to B. oleracea and B. rapa (Jeong et al., 2016). Both Raphanus and Brassica belong to the Brassicaceae family, whose divergence was estimated at 7-14 million years ago (Mya) after recent whole-genome triplication (WGT) event in a common hexaploid ancestor (Cheng et al., 2017; Jeong et al., 2016). It has been suggested that the basal chromosome number of nine in radish was derived from the diploid ancestor with seven chromosomes, and the proto-Raphanus karyotype with 11 chromosomes underwent WGT event (Cheng et al., 2017). In this study, a relatively weak syntenic relationship between radish and the two Brassica genomes was observed, despite the radish and B. oleracea have a common 9 haploid chromosome. The complex patterns of conserved synteny were accordant well with previous finding that the Brassica chromosomes have occurred extensive chromosomal breakages and fusions, which was probably a result of mesopolyploidy genome in Brassica (Liu et al., 2014). Previous studies demonstrated that the chromosome 1 and chromosome 2 between B. rapa and B. oleracea displayed high level of collinearity by comparative genomic analyses (Chalhoub et al., 2014). In this study, radish LG1 and LG2 were matched to three chromosomes of B. rapa and B. oleracea, illustrating the more chromosomal rearrangements resulted in a faster rate of genome evolution in radish. Taken together, our results provide insights into the comparative and evolutionary genomics research between the radish and the two Brassica species.

Recombination contributed to phenotypic variation and breeding applications

Meiotic recombination is a fundamental biological process that generates genetic variation and new allele combinations, which have a critical role in phenotypic variations and genome evolution.
The high-resolution genetic map reported in this study provides a genome-wide recombination ratio of 3.8 cM/Mb, which is in the similar range as can be calculated from Arabidopsis (Drouaud et al., 2006) and rice (Wu et al., 2003), but is higher than earlier estimates from cotton (1.8 cM/Mb) (Wang et al., 2015) and maize (0.9 cM/Mb) (Pan et al., 2016). Recombination varied across all the chromosomes and displayed a low recombination at telomeres, which are known to be highly heterochromatic (Wang et al., 2015). Some studies have reported that a higher recombination rate was observed in domesticated plants species as compared with their wild progenitors, illustrating that the high recombination rates are an adaptation favoured under artificial selection (Ritz et al., 2017; Si et al., 2015). The 504 RHRs identified in radish genome may help to facilitate marker-assisted selection and shorten the breeding period by construction of populations with a higher recombination rate in specific genome regions.

Previous studies have showed that recombination rates are increased near annotated TSSs and TTSs and decreased within the transcribed region in animals (Auton et al., 2012; Singhal et al., 2015) and plants such as A. thaliana (Choi et al., 2013). In the present study, the increase in recombination rates near TSSs and TTSs supports the viewpoint mechanism that the recombination occurs at TSSs and TTSs is very close to the recombination machinery (Singhal et al., 2015). The density of disease resistance genes showed a significant positive correlation with the recombination rates in the rice genome (Si et al., 2015). The intragenic recombination has a potential effect on phenotypic variation in maize (Pan et al., 2016). These findings indicated that the frequency and distribution of recombination plays an important role in influencing the progress of breeding programme.

The high recombination rate was observed in rapidly environment changes (Ritz et al., 2017). In the current study, the genes within RHRs showed the significantly enriched GO terms associated with regulation of metabolic process and organic substance metabolic process in biological processes. These results were consistent with the observation that plant metabolism contributes to face unavoidable environmental changes (Silva-Junior and Grattapaglia, 2015). In sharp contrast to rice, frequent recombination primarily involved in response to environmental stimuli (Si et al., 2015). Accordingly, adaptation in a variable environment with different strategies might exist in plants and a maximize recombination rate would be adopted for populations under selection. Many studies showed that the regulatory region containing recombination is significantly related to quantitative variation in expression level and generated adaptive genetic variations during crop domestication and improvement (Pan et al., 2016; Si et al., 2015). Also, the intragenic recombination had potential effects on gene expression and phenotypic variation in maize (Pan et al., 2016). It was found that recombination events were occurred in three QTLs, and Rs407980 for root length involved in metabolic process was associated with recombination hotspot. These results could provide fundamental insight into complex landscape of recombination in radish and act as a reference to investigate recombination rate variation in Brassicaceae.

In conclusion, a high-density genetic map with high resolution and accuracy was constructed through whole-genome sequenc- ing. A candidate causal gene for taproot red skin was identified using a combination of genome-based QTL mapping and map-based cloning. RsMYB90 gene plays an important role in regulating anthocyanin biosynthesis, and the identification of RsMYB90 is useful for further genetic manipulation of antho-
cyanin accumulation in radish. The high-density genetic map was applied for comparative genomic study and the exploration of recombination landscape at whole-genome scale in radish, which would provide a robust basis for further investigation on genetic mapping and genomic recombination and facilitate further molecular breeding in radish.

Experimental procedures

Plant materials and phenotype data collection

Two advanced radish inbred lines, ‘NAU-LB’ with white taproot skin and ‘NAU-YH’ with the red taproot skin, were used in this study. The ‘NAU-LB’ (P1) was crossed with ‘NAU-YH’ (P2) to produce the F1, F2, BC1P1 and BC1P2 populations. The F2 populations consisted of 137 individuals were used for linkage analysis and genetic map construction. A F2 population (430 lines) derived from the same cross was developed for fine mapping of genes associated with root skin colour. All the plants were grown in the JiangPu Breeding Station of Nanjing Agricultural University. To dissect the genetic mechanism underlying important horticultu- re traits in radish, the phenotypic data of ten traits, including root length, root weight and plant height, were measured from the F2 individuals and their two parental lines. The pigment phenotypes of root skin were investigated visually. For genetic analysis, chi-square tests (χ2) were conducted to evaluate the goodness of fit of segregation ratios in F2, BC1P1 and BC1P2 populations.

Population resequencing and genotyping

The genomic DNA of the two parents and F2 plants was extracted from the fresh leaf tissue following the CTAB (cetyl trimethylammonium bromide) method with minor modifications (Liu et al., 2003). The libraries were constructed with an insert size of 180 bp and sequenced on the Iillumina HiSeq 2500 platform according to the manufacturer’s standard protocols. Reads with ≥10% unidentified nucleotides and >50% bases with Phred quality <5 were filtered before alignment. The sequencing reads were aligned to the radish reference genome (‘WK100039’ http://radish-genome.org/) using Burrows–Wheeler Aligner (BWA) tools with the parameters of ‘mem -t 4 -k 32 -M -R’ (Li and Durbin, 2009). Alignment files were converted into BAM files using the sort setting in SAMTools software (Li et al., 2009). Only the reads mapped uniquely to the reference genome sequence were then used to call SNPs. Identification of SNPs between the parental lines and F2 individuals was performed with the GATK software (McKenna et al., 2010). Polymorphic markers between the two parental lines with aa × bb segregation pattern were retained and genotyped for F2 individuals. High-quality SNPs were obtained with a custom Perl script by the following criteria: the minimum sequencing depth of each SNP allele was 4, and an allele represented at least 30% F2 plants with SNPs.

Phenotype evaluation

The root skin colour was defined as red or white. The leaf number (LN) was measured by artificial statistics. Plant height (PH), leaf width (LW), root length (RL) and root diameter (RD) were measured using Vernier calipers. Plant weight (PW) and root weight (RW) were measured using electronic scales. The content of total soluble solid content (TSS) was measured using a refractometer (Atago, model N-1). The content of soluble sugar (SS) and soluble protein (SP) was measured as described by Su et al. (2016).
Determination of total anthocyanin content

Total anthocyanins were extracted from 0.3 g radish taproot skin using the pH differential method described by Jiang et al. (2016). The absorbance of the solutions was measured by a multimode microplate reader (Infinite M200, Tecan Inc., Manndorf, Switzerland). The anthocyanin content was calculated with the following formula: TA = A × MW × S × 100 × W/f, where TA is the total anthocyanin content (mg/100 g fresh weight), A = [A510 nm (pH 1.0) − A700 nm (pH 1.0)] − [A510 nm (pH 4.5) − A700 nm (pH 4.5)], MW stands for molecular weight of cyanidin-3-glucoside (MW = 449.2), S stands for final volume (mL), and c is the extinction coefficient of cyanidin-3-glucoside (c = 26 900). The analyses of each sample were performed in triplicate.

Bin map construction

Chi-square test was conducted to detect the ratio of marker segregation, and markers with significantly distorted segregation (P-value < 0.001) were filtered for further analysis. A slightly modified sliding window approach was adopted to calculate the ratio of SNP alleles derived from ‘NAU-LB’ and ‘NAU-YH’ and identified recombinant breakpoints (Huang et al., 2009). A window size of 15 SNPs with no missing was used for genotyping calling. Windows with 11 or more SNPs from either parent were considered to be homozygous for an individual, while those with less were called as heterozygous. Consecutive 100-kb intervals considered to be homozygous for an individual, while those with white skin from combined F2 population (Table S10). Linkage analysis was conducted using JoinMap 4.0 with an LOD threshold score of 6.0.

Genome synteny and characterization of RHRs

MCScanX was employed to detect syntic regions (Wang et al., 2012) and consensus sequences of all mapped SNP markers were searched against the genome sequences of R. sativus, B. rapa and B. oleracea using BLASTX with a cut-off E-value < 1.0E-10. Marker with multiple hits was filtered, and only the best hits were employed. The graphical comparative maps were visualized using Circos program (http://circos.ca/).

The estimation of recombination rate (cM/Mb) was calculated using in-house Perl scripts, which divided the genetic length of the segment in cM by the physical length of the segment in Mb. The bin marker interval with recombination rate greater than 1.0 cM/Mb was defined as RHRs. The relationships between recombination rate within RHRs and genomic features (SNP density, gene density and GC content) were tested. The overlapping rate of RHRs with exons, introns, 5’UTRs, 3’UTRs, 1 kb TSS and 1 kb TTS was measured. The randomly permuted genomic regions were assessed for their overlap with each feature. The genes overlapped RHRs were conducted to perform gene ontology (GO) category enrichment analysis.

Quantitative real-time PCR (RT-qPCR) analysis

Total RNA was extracted from root skin of ‘NAU-YH’ and ‘NAU-LB’ using the TRIzol Reagent (Invitrogen, Carlsbad, CA, USA) kit. cDNA was synthesized using the SuperScript III First-Strand Synthesis System (Invitrogen). The PCR and amplification were performed as described previously (Xu et al., 2015a). RT-qPCR analysis was carried out with SYBR Green PCR Master Mix (TaKaRa) on a ROCHE LightCycler 480 instrument according to the manufacturer’s instructions. Reactions were run with three biological replicates. The radish Actin gene was used as an internal control. The relative expression of each target gene was normalized with the 2^(-ΔΔC_T) algorithm (Livak and Schmittgen, 2001). All the primer sequences are listed in Table S10.

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Conflict of interest

The authors declare no competing financial interests.
Author' contributions
X.L. performed the data analysis and wrote the manuscript. M.T. and J.D. conducted validation of experiments. L.F. and Y.W. contributed powerful analytical tools. Y.C and Y.Z. helped with the revise of the manuscript. L.L. and L.X. conceived and designed the research. All authors read and approved the final manuscript.

References
Abe, A., Kosugi, S., Yoshida, K., Natsume, S., Takagi, H., Kanzaki, H., Matsumura, H. et al. (2012) Genome sequencing reveals agronomically important loci in rice using MutMap. Nat. Biotechnol. 30, 174–178.
Allan, A.C., Hennens, R.P. and Laing, W.A. (2008) MYB transcription factors that colour our fruit. Trends Plant Sci. 13, 99–102.
Auton, A., Fledel-Alon, A., Pfeifer, S., Venn, O., Ségurel, L., Street, T., Leffler, E.M. et al. (2012) A fine-scale chimpanzee genetic map from population sequencing. Science, 336, 193–198.
Ban, Y., Honda, C., Hatsuyama, Y., Igarashi, M., Bessho, H. and Moriguchi, T. (2009) Molecular mapping in oil radish (Raphanus sativus L.) and QTL analysis of resistance against beet cyst nematode (Heterodera schachtii). Theor. Appl. Genet. 118, 775–782.
Cao, J., Schneeberger, K., Ossowski, S., Günther, T., Bender, S., Fitz, J., Koenig, D. et al. (2011) Whole-genome sequencing of multiple Arabidopsis thaliana populations. Nat. Genet. 43, 956–963.
Chalhoub, B., Denoeud, F., Liu, S.Y., Parkin, I.A., Tang, H.B., Wang, X.Y., Cao, J., Schneeberger, K., Ossowski, S., Budahn, H., Peterka, H., Mousa, M.A.A., Ding, Y.H., Zhang, S.S. and Li, J.B. (2010) Cloning of the papaya chromoplast-specific lycopene β-cyclase, CpCYC-b, controlling fruit flesh color reveals conserved microsynteny and a recombination hot spot. Plant Physiol. 152, 2013–2022.
Drouaud, J., Camilleri, C., Bourguignon, P.Y., Canaguier, A., Beltcheva, I. and Chiquet, J. (2009) Molecular mapping in oil radish (Raphanus sativus L.) and QTL analysis of resistance against beet cyst nematode (Heterodera schachtii). Theor. Appl. Genet. 118, 775–782.
Fu, W.Q., Chen, D.Z., Pan, Q., Li, F.F., Zhao, Z.G., Ge, X.H. and Li, Z.Y. (2017) Production of red-flowered oilseed rape via the ectopic expression of Orychophragmus violaceus OsPP2C. Plant Biotechnol. J. 15, 1372–1379.
Gao, Z.Y., Zhao, S.C., He, W.M., Guo, L.B., Peng, Y.L., Wang, J.J., Guo, X.S. et al. (2013) Dissecting yield-associated loci in super hybrid rice by resequencing recombinant inbred lines and improving parental genome sequences. Proc. Natl Acad. Sci. USA, 110, 14492–14497.
Gonzalez, A., Zhao, M., Leavitt, J.M. and Lloyd, A.M. (2008) Regulation of the anthocyanin biosynthetic pathway by the TGT1/HHLMyb transcriptional complex in Arabidopsis seedlings. Plant J. 53, 814–827.
Grotewold, E. (2006) The genetics and biochemistry of floral pigments. Annu. Rev. Plant Biol. 57, 761–780.
He, J. and Giusti, M.M. (2010) Anthocyanins: natural colorants with health-promoting properties. Annu. Rev. Food Sci. Technol. 1, 163–187.
Hu, Z.Y., Deng, G.C., Mou, H.P., Xu, Y.H., Chen, L., Yang, J.H. and Zhang, M.F. (2017) A re-sequencing-based ultra-dense genetic map reveals a gummy stem blight resistance-associated gene in Cucumis melo. DNA Res. 25, 1–10.
Huang, X.H., Feng, Q., Qian, Q., Zhao, Q., Wang, L., Wang, A.H., Guan, J.P. et al. (2009) High-throughput genotyping by whole-genome resequencing. Genome Res. 19, 1068–1076.
Hunter, C.M., Huang, W., Mackay, T.F. and Singh, N.D. (2016) The genetic architecture of natural variation in recombination rate in Drosophila melanogaster. PLoS Genet. 12, e1005951.
Ilk, N., Ding, J., Hnatowicz, A., Koornneef, M. and Reymond, M. (2015) Natural variation for anthocyanin accumulation under high-light and low-temperature stress is attributable to the ENHANCER OF AG-4-2 (H2A.Z) locus in combination with PRODUCTION OF ANTHOCYANIN PIGMENT1 (PAP1) and PAP2. New Phytol. 206, 422–435.
Jaakola, L. (2013) New insights into the regulation of anthocyanin biosynthesis in fruits. Trends Plant Sci. 18, 447–483.
Jaakola, L. (2013) New insights into the regulation of anthocyanin biosynthesis in fruits. Trends Plant Sci. 18, 447–483.
Jeong, Y.M., Kim, N., Ahn, B.O., Oh, M., Chung, W.H., Chung, H., Lim, K.B. et al. (2016) Elucidating the triplicated ancestral genome structure of radish based on chromosome-level comparison with the Brassica genomics. Theor. Appl. Genet. 129, 1357–1372.
Jiang, M.M., Ren, L., Lian, H.L., Liu, Y. and Chen, H.Y. (2016) Novel insight into the mechanism underlying light-controlled anthocyanin accumulation in eggplant (Solanum melongena L.). Plant Sci. 249, 46–58.
Jaakola, L. (2013) New insights into the regulation of anthocyanin biosynthesis in fruits. Trends Plant Sci. 18, 447–483.
Koebes, R., Verweij, W. and Quattrocchio, F. (2005) Flavonoids: a colorful model for the regulation and evolution of biochemical pathways. Trends Plant Sci. 10, 236–242.
King, Q., Pattanaik, S., Feller, A., Werkman, J.R., Chai, C., Wang, Y., Grotewold, E. et al. (2012) Regulatory switch enforced by basic helix-loop-helix and ACT-domain mediated dimerizations of the maize transcription factor R. Proc. Natl Acad. Sci. USA, 109, E2091–E2097.
Li, H. and Durbin, R. (2009) Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics, 25, 1754–1760.
Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G. et al. (2009) The sequence alignment/map format and SAMtools. Bioinformatics, 25, 2078–2079.
Li, L.T., Deng, C.H., Knabel, M., Chagné, D., Kumar, S., Sun, M.J., Zhang, S.L. et al. (2017) Integrated high-density consensus genetic map of Pyrus and anchoring of the ‘Barlett’ v.1. 0 (Pyrus communis) genome. DNA Res. 24, 289–301.
Liu, L., Guo, W., Zhu, X. and Zhang, T. (2003) Inheritance and fine mapping of fertility-restoration for cytoplasmic male sterility in Gossypium hirsutum L. Theor. Appl. Genet. 106, 461–469.
Li, S.Y., Liu, Y.M., Yang, X.H., Tong, C.B., Edwards, D., Parkin, I.A.P., Zhao, M.X. et al. (2014) The Brassica oleaceae genome reveals the asymmetrical evolution of polyploid genomes. Nat. Commun. 5, 3930.
Liak, K.J. and Schmitz, T.D. (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2^-Delta Delta CT method. Methods, 25, 402–408.
Li, X., Xiong, Q., Cheng, T., Li, Q.T., Liu, X.L., Bi, Y.D., Li, W. et al. (2017) A PP2C-F1 allele underlying a quantitative trait locus enhances soybean 100-seed weight. Mol. Plant. 10, 670–684.
McKenna, A., Hanna, M., Banks, E., Skafchenko, A., Cibulskis, K., Kernytsky, A., Garimella, K. et al. (2010) The genome analysis toolkit: a mapreduce
framework for analyzing next-generation DNA sequencing data. Genome Res. 20, 1297–1303.

Mitsuji, Y., Shimomura, M., Komatsu, K., Namiki, N., Shibata-Hatta, M., Imai, M., Katayose, Y. et al. (2015) The radish genome and comprehensive gene expression profile of tuberous root formation and development. Sci. Rep. 5, 10835.

Moghe, G.D., Hufnagel, D.E., Tang, H.B., Xiao, Y.L., Dworkin, I., Town, C.D., Conner, J.K. et al. (2014) Consequences of whole-genome tripllication as revealed by comparative genomic analyses of the wild radish Raphanus raphanistrum and three other Brassicaceae species. Plant Cell. 26, 1925–1937.

Mun, J.H., Chung, H., Chung, W.H., Oh, M., Jeong, Y.M., Kim, N., Ahn, B.O. et al. (2015) Construction of a reference genetic map of Raphanus sativus based on genotyping by whole-genome resequencing. Theor. Appl. Genet. 128, 259–272.

Nabavi, S.F., Habtemariam, S., Daglia, M., Shafighi, N., Barber, A.J. and Nabavi, S.M. (2015) Anthocyanins as a potential therapy for diabetic retinopathy. Curr. Med. Chem. 22, 51–58.

Pan, Q.C., Li, L., Yang, X.H., Tong, H., Xu, S.T., Li, Z.G., Li, W.Y. et al. (2016) Genome-wide recombination dynamics are associated with phenotypic variation in maze. New Phytol. 201, 1083–1094.

Ritz, K.R., Noor, M.A.F. and Singh, N.D. (2017) Variation in recombination rate: adaptive or not? Trends Genet. 33, 364–374.

Si, W.N., Yuan, Y., Huang, J., Zhang, X.H., Zhang, Y.C., Zhang, Y.D., Tian, D.C. et al. (2015) Widely distributed hot and cold spots in meiotic recombination as shown by the sequencing of rice F2 plants. New Phytol. 206, 1491–1502.

Silva-Junior, O.B. and Grattapaglia, D. (2015) Genome-wide patterns of recombination, linkage disequilibrium and nucleotide diversity from pooled resequencing and single nucleotide polymorphism genotyping unlock the evolutionary history of Eucalyptus grandis. New Phytol. 208, 830–845.

Singhal, S., Jeffreys, E.M., Sannareddy, K., Turner, I., Venn, O., Hooper, D.M., Strand, A.J. et al. (2015) Stable recombination hotspots in birds. Science, 350, 928–932.

Su, N., Wu, Q. and Cui, J. (2016) Increased sucrose in the hypocotyls of radish Raphanus sativus revealed by comparative genomic analyses of the wild radish Raphanus raphanistrum and three other Brassicaceae species. Plant Cell. 26, 1925–1937.

Tsuro, M., Suwabe, K., Kubo, N., Matsumoto, S. and Hirai, M. (2008) Mapping of QTLs controlling root shape and red pigmentation in radish, Raphanus sativus L. Theor. Appl. Genet. 125, 659–670.

Yu, X.Y., Zeng, L., Tao, Y., Vuong, T., Wan, J.R., Boerma, R., Nee, J. et al. (2013) Pinpointing genes underlying the quantitative trait loci for root-knot nematode resistance in palaeopolyploid soybean by whole genome resequencing. Proc. Natl Acad. Sci. USA, 110, 13469–13474.

Xie, X.B., Li, S., Zhang, R.F., Zhao, J., Chen, Y.C., Zhao, Q., Yao, Y.X. et al. (2015a) De novo sequencing of root transcriptome reveals complex cadmium responsive regulatory networks in radish (Raphanus sativus L.). Plant Sci. 236, 313–323.

Xu, W., Dubos, C. and Lepinejac, L. (2015b) Transcriptional control of flavonoid biosynthesis by MYB-bHLH-WDR complexes. Trends Plant Sci. 20, 176–185.

Yamagishi, M., Toda, S. and Tasaki, K. (2014) The novel allele of the rMyB12 gene is involved in splatter-type spot formation on the flower tepals of Asiatic hybrid ilies (Lilium spp.). New Phytol. 201(3), 1009–1020.

Yao, G.F., Ming, M.L., Allan, A.C., Gu, C., Li, L.T., Wu, X., Wang, R.Z. et al. (2017) Map-based cloning of the pear gene MYB114 identifies an interaction with other transcription factors to coordinately regulate fruit anthocyanin biosynthesis. Plant J. 92, 437–451.

Ye, J., Wang, X., Hu, T.X., Zhang, F.X., Wang, B., Li, C.X., Yang, T.X. et al. (2017) An inbred in the promoter of Al-activated Malate Transporter9 selected during tomato domestication determines fruit malate contents and aluminum tolerance. Plant Cell. 29, 2249–2268.

Yu, X., Wang, H., Zhong, W.L., Bai, J.J., Liu, P.L. and He, Y.K. (2013) QTL mapping of leafy heads by genome resequencing in the RIL population of Brassica rapa. PLoS ONE, 8, e76059.

Yu, X.N., Choi, S.R., Dhandapani, V., Ramenemi, J.J., Li, X.N., Pang, W.X., Lee, J.Y. et al. (2016) Quantitative trait loci for morphological traits and their association with functional genes in Raphanus sativus. Front. Plant Sci. 7, 255.

Yuan, Y.X., Chiu, I.W. and Li, L. (2009) Transcriptional regulation of anthocyanin biosynthesis in red cabbage. Planta, 239, 1141–1153.

Yu, W.Y., Rebocho, A.B., Sagawa, J.M., Stanley, I.E. and Bradshaw, H.D. (2016) Competition between anthocyanin and flavonol biosynthesis produces spatial pattern variation of floral pigments between Mimulus species. Proc. Natl Acad. Sci. USA, 113, 2448–2453.

Zhang, Q.L., Ma, C., Zhang, Y., Gu, Z.Y., Li, W., Duan, X.W., Wang, S.N. et al. (2018) A single nucleotide polymorphism in the promoter of a hairpin RNA contributes to Alternaria alternata leaf spot resistance in apple (Malus × domestica borkh.). Plant Cell, 30, 1924–1942.

Zou, Z., Ishida, M., Li, F., Kakizaki, T., Suzuki, S., Kitabishi, H. and Nishio, T. (2013) QTL analysis using SNP markers developed by next-generation sequencing for identification of candidate genes controlling 4-methylthio-3-butenyl glucosinolate contents in roots of radish, Raphanus sativus L. PLoS ONE, 8, e53541.

Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1 A high-density linkage map with bin markers of radish.

Figure S2 Distribution of distorted segregating bins across the radish genome.

Figure S3 Frequency distribution of ten traits in F2 population of radish.

Figure S4 Interval mapping analysis on the LG7 for red skin colour of radish taproot.

Figure S5 Expression profiling of RmMYB90 in taproot of two parental lines (a,b) and different genotypes (c).

Figure S6 Phylogenetic tree of RsMYB90 and R2R3 MYBs of other plant species.
Figure S7 Protein sequence alignment of RsMYB90 and R2R3 MYB proteins from different species.
Figure S8 Genetic versus physical distance maps of radish nine chromosomes.
Figure S9 The relationship between recombination rate and physical length of the radish chromosomes.
Figure S10 Gene ontology enrichment of the genes within recombination hot regions (RHRs).
Figure S11 Amino acid sequence (a) and promoter sequence (b) of RsMYB90 gene in the two parental lines.

Table S1 Statistics of sequencing data.
Table S2 The distribution of bin markers in linkage group.
Table S3 Segregation distortion markers in F2 population at a significance level of $P < 0.005$.
Table S4 The distribution of segregation distortion regions.
Table S5 Correlation coefficients among ten traits in radish.
Table S6 QTLs analysis for ten traits in radish.
Table S7 The statistics analysis of the sequencing data in R-bulk and W-bulk.
Table S8 Gene prediction for the candidate interval.
Table S9 The location of recombination hot regions (RHRs) on the radish chromosome.
Table S10 Primer sequences used in this study.