Identification of quantitative trait loci controlling radish root shape using QTL-seq

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Abstract

Root shape is an important characteristic that affects the commodity of radish (Raphanus sativus L.). This trait can be measured using the ratio of root length (RL) to root diameter (RD). Although it is known that root shape is controlled by multiple quantitative trait loci (QTLs), reliable QTLs for regulating radish root shape are still lacking. In the present study, three F₂ populations were constructed by crossing five radish cultivars with significant differences in root shape. A total of 1282 F₂ individuals were measured to determine the root length and maximum diameter. High depth resequencing of six extreme pools and five parents was performed, and the bulk segregant approach coupled with whole genome re-sequencing (QTL-seq) was used to detect QTLs. The results showed that radish root shape was positively correlated with root length. Seven QTLs distributed on five radish chromosomes (R1, R2, R4, R5, and R7) were identified for root shape, two of which (rs7.1 and rs7.2) had an overlap of 1.02 Mb (13.79-14.81 Mb). In addition, rs4.1 and rs4.2 were adjacent to each other on the R4 chromosome. The present study provides an important foundation for the fine mapping and functional analysis of root shape QTLs as well as cultivar breeding for root shape.

Key Message

Seven QTLs for radish root shape were detected distributed on five chromosomes using QTL-seq method with three F₂ populations.

Introduction

Radish (Raphanus sativus L.), belonging to the Brassicaceae, is an important root vegetable crop, especially in East Asia. The radish root is generally composed of the upper part which develops from the hypocotyls, and the lower part consisting of true root tissue (Masato Tsuro et al. 2008). The thick and succulent root can be cooked, processed with salt, or consumed fresh. The diverse consuming habits of radish root in different regions result in demands for different root shapes. The shapes of roots directly affect the yield and quality of radish, thus root shape is an important target trait in radish breeding (Hiroyoshi I et al. 2004). The root shape of radish can be measured using the ratio of the root length (RL) to the root diameter (RD) at the maximum part of the root, which displays abundant diversity (Masato Tsuro et al. 2008). Based on radish germplasm resources, the diameter of radish roots ranges from 1 to 30 cm, whereas the length of the root ranges from 3 to 200 cm (Mitsui et al. 2015). In general, the fleshy roots of radish are divided into 15 main shapes, including cylindrical, conical, oval, oblate, pear-shaped, and round (Li XX et al. 2008).

The shape of radish roots is a quantitative trait. However, QTLs for root shape have rarely been found. Radish roots are controlled by complex genetic, environmental, and physiological factors (Zaki HEM et al. 2010). Tsuro et al. (Masato Tsuro et al. 2008) constructed a genetic map containing 198 markers using isolated F₂ populations and detected three QTLs associated with the root shape index, accounting for 42.4% of the phenotypic variation. At the same time, two QTLs associated with root diameter were detected, among which the QTL on linkage group 8 affected root shape by affecting diameter (Masato Tsuro et al. 2008). Hashida et al. identified three QTLs associated with fleshy root weight using a recombinant inbred line (RIL) population combined with two-year trait data (Hashida T et al. 2013). At present, there are few studies on gene mapping
of the fleshy root shape traits, whereas QTL mapping for radish root shape is in the preliminary stage. Effective molecular markers closely linked to fleshy root traits are still not available. Most of the research on the development of fleshy radish roots focus on transcriptome studies at different developmental stages (Wang S et al. 2012; Mitsui Y et al. 2015; Yu R et al. 2016), and a number of genes across different developmental stages of fleshy radish roots have been identified. However, the genes directly regulating the root shape of radish remain to be found and verified, and the molecular mechanisms of fleshy root enlargement have not been elucidated.

QTL-seq has been successfully applied to identify quantitative traits and extreme traits in rice, which has been widely used in many crops such as tomato, and cucumber (Takagi H et al. 2013; Lu H et al. 2014; Illa-Berenguer E et al. 2015; Anurag D et al. 2016; Wei Q.Z et al. 2016). This method has also been used to detect the genes for root cuticular and interior color in radish (Liu T et al. 2019; Wang Q et al. 2020). Genome-wide single nucleotide polymorphism (SNP) analysis allowed the detection of a genomic region harboring the major QTL, whereas QTL-seq provided a cost-effective and time-efficient method for the identification of multiple QTLs.

With the development of sequencing technology and the reduction in sequencing costs, the genomic sequences of five radishes have been published (Kitashiba H et al. 2014; Moghe, GD et al. 2014; Mitsui Y et al. 2015; Zhang XH et al. 2015; Shirasawa K et al. 2020). The quality of the radish genome is in continuous improvement, the latest version of which uses single-molecule real-time sequencing technology, with a genome contig N50 of 1.2 Mb. The improvement in the genomic integrity greatly facilitates the detection of key genes involved in the formation of fleshy roots of different shapes. In the present study, we performed QTL-seq combined with bulk-segregant approach using three F₂ populations to identify QTLs controlling root shape of radish. The results provide an important basis for the development of closely linked molecular markers and radish cultivar improvement on root shape.

**Materials And Methods**

**Plant materials**

Three F₂ populations derived from independent crosses were developed and evaluated regarding their root shape index (Fig. 1; Table 1). A total of five parents were used, among which CZ was leaf radish without enlarged fleshy roots. LLYH was a small oblate radish with a fleshy root length of 3.0 cm and a maximum diameter of 3.5 cm at normal maturity. CLA was a large long white radish, with fleshy roots that were oblate, long and conical, had a normal maturity length of approximately 50.0 cm and had a maximum diameter of approximately 8.0 cm. R05 was a small oval radish with a normal maturity length of 5.5 cm and a maximum diameter of 5.0 cm. BY was a slender white radish with a normal maturity length of approximately 60.0 cm and a maximum diameter of approximately 7.0 cm. The root length and diameter values mentioned above are approximate values corresponding to the normal commercial maturity stage of radish.

**Phenotypic analysis**
Since the growth period of the parents of the F\textsubscript{2} population is quite different, the growth and maturity periods of each single F\textsubscript{2} plant were expected to be different. However, to facilitate the statistical analysis of the phenotypic data of radish roots grown underground, we chose the same growth days for the measurement of phenotypic data. The parental, F\textsubscript{1} and F\textsubscript{2} plants were grown in fall 2018 at the Qiaosi experimental field of Zhejiang Academy of Agricultural Sciences, Hangzhou, China. The seeds were sown on September 28. They were spaced 25.0 cm apart with ridges spaced 50.0 cm apart and grown under natural conditions. The radishes were pulled up and measured on November 12 at a consistent time (Masato Tsuro et al. 2008). The root length and the maximum root diameter were measured regardless of the growth cycle and the fact that a consistent growth time of 45 d was used, since the growing stages did not affect the root shape characteristic (Hiroyoshi I et al. 2004). The RL/RD ratio was calculated as the root shape index value. All the measurements were made with 30.0 cm digital display Vernier calipers.

**DNA isolation and sequencing of pooled samples**

The young leaves of the parents and F\textsubscript{2} plants were sampled, and the samples were frozen with liquid nitrogen and stored in a -80 °C cryogenic refrigerator for later use. According to the calculated value of the root shape index, bulks containing approximately 10% of the individual plants with extreme traits were selected for QTL-seq from each F\textsubscript{2} population, except the 1902 population. The modified CTAB method was used to extract DNA from each selected plant (Samuolien G et al. 2011). The quality of the DNA was detected by agarose gel electrophoresis, and the concentration of the DNA was simultaneously detected by NanoDrop. Then, the sample DNA was combined based on equal concentrations, and the small root shape index pool (S) and large root shape index pool (L) were formed. DNA samples from the parents and extreme pools were sequenced using the Illumina sequencing technology platform.

**QTL-seq analysis**

Using the newly published radish genome as a reference genome (Shirasawa et al. 2020), BWA software was used to compare and localize the clean reads to the reference genome (Choi EY et al. 2011). Then, we used Picard analysis for repeat sequences (http://sourceforge.net/projects/picard/) to ensure the accuracy of the SNPs detected by GATK (Yu RG et al. 2015). Partial rematching and base mass value correction were performed, GATK was used for SNP detection and filtering, and the final SNP data were obtained. The filtering of the SNPs was carried out as follows: SNPs with multiple genotypes were removed, then SNPs with a read support value of less than four were removed, and finally, SNPs with identical genotypes between the mixed pools and SNPs with recessive alleles in the mixed pools but not the parents were filtered out. Finally, high-quality credible SNPs were obtained. According to the QTL-seq method, we calculated the SNP index using a 1 Mb sliding window and a 10 kb for walking window (Takagi H et al. 2013), then, we calculated the average value in each sliding window using the DISTANCE method to fit the Δ SNP index.

**Gene annotation of QTL regions**

SNPEFF software was used to annotate the variation in the SNPs located in QTL regions (Geupil J et al. 2015). According to the regional variation in loci and the gene position information from the reference genome, the SNPs occurring in the intergenic regions, gene regions or CDS regions were analyzed and
classified as synonymous mutations or nonsynonymous mutations. The GO and KEGG databases were annotated in depth using BLAST software (Murray M et al. 1980; Li H et al. 2009; Muvva C et al. 2012).

Results

Phenotypic data analysis of the radish root shape characteristics

Three F₂ populations (1902, 1908, and 1909) were constructed by the following crosses: CZ×CLA, R05×CLA, LLYH×BY, respectively. Phenotypic data was collected from the three F₂ populations and six parents. The growth periods of the parental rad-ishes showed relatively large differences. For example, compared with the large long white radish CLA which usually needs ~80 days to get mature, the small oval radish LLYH only needs ~35 days; whereas BY needs ~70 days to be mature and R05 needs ~50 days. Previous study showed that root shape can be efficiently selected even 40 days after sowing and breeding efficiency may improve by starting selection at an early growth stage (Hiroyoshi I et al. 2004). Combined with the maturing period of the five radish parents, the three F₂ populations and parents were harvested and measured at 45 days of growth. The photographs of the parents at normal maturity and the extreme F₂ plants at 45 days of growth are shown in Fig. 1. The phenotypic data of the parents at 45 days of growth and the sequencing information from the mixed pools are collected (Table 1 and Table S1). The root length ranged greatly among the F₂ individuals and parents, from 2.87cm to 20.10cm. On the contrast, the root diameter ranged slightly from 3.46cm to 5.18 cm. The root shape index varied from 0.82 to 5.52. The largest root shape index was found for BY, with an average value of 5.52, followed by CLA at 4.82, LLYH at 0.82, and R05 at 1.01. Unlike the rest radish parents which are usually consumed as root vegetables, CZ is mainly consumed as a leaf vegetable without enlarged fleshy roots. Thus no phenotypic data were collected for root characteristics (Fig. 1; Table 1). Notably, although one parent of the F₂ population 1902 was a leaf radish, most individuals of the F₂ plants formed enlarged fleshy roots (Table S1).

Table 1. Phenotypic data of the parents at 45 days of growth.

<table>
<thead>
<tr>
<th>F₂ Population</th>
<th>Parent</th>
<th>Root length/cm</th>
<th>Root diameter/cm</th>
<th>Root shape index</th>
</tr>
</thead>
<tbody>
<tr>
<td>1902</td>
<td>CZ</td>
<td>/</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td></td>
<td>CLA</td>
<td>18.66±0.37</td>
<td>3.87±0.09</td>
<td>4.82±0.03</td>
</tr>
<tr>
<td>1908</td>
<td>R05</td>
<td>5.23±0.39</td>
<td>5.18±0.39</td>
<td>1.01±0.09</td>
</tr>
<tr>
<td></td>
<td>CLA</td>
<td>18.66±0.37</td>
<td>3.87±0.09</td>
<td>4.82±0.03</td>
</tr>
<tr>
<td>1909</td>
<td>LLYH</td>
<td>2.87±0.06</td>
<td>3.46±0.17</td>
<td>0.82±0.04</td>
</tr>
<tr>
<td></td>
<td>BY</td>
<td>20.10±0.51</td>
<td>3.64±0.08</td>
<td>5.52±0.05</td>
</tr>
</tbody>
</table>

Inheritance of the root shape index and mixed pools
The frequency distribution of the root shape index in three F\textsubscript{2} populations in radish was calculated. The root shape index in all the F\textsubscript{2} populations showed a normal distribution (Fig. 2), suggesting that the root shape index were inherited as a quantitative trait controlled by multiple genes (Masato Tsuro et al. 2008). The phenotypic data for root length and diameter from 1282 F\textsubscript{2} individuals were statistically analyzed (Table S1), among which 432 plants were from the F\textsubscript{2} population 1902, 397 plants were from the F\textsubscript{2} population 1908, and 453 plants were from the F\textsubscript{2} population 1909. Among them, approximately 10% of the individual plants with extreme phenotypes were selected for mixed pool sequencing for populations 1908 and 1909. For population 1902, because one of the parents was a leaf radish and most of the F\textsubscript{2} individuals had more fibrous roots, the number of individual plants in the mixed sequencing pools was reduced, accounting for approximately 8% of the population. In the 1902 population, the value of the root shape index ranged from 0.608 to 4.109. The 1909 population has the largest root index value of 4.468. In the 1908 population, the range of the root shape index was 0.847-3.643, which was smaller than that in the 1902 and 1909 populations. However, the minimum mean value of the three extremely small pools was 0.893 in the 1909 population, and the maximum mean value of the three extremely large pools was 3.253 in the 1902 population (Table 2).

Table 2 Information on the mixed pools and phenotypic data for the three F\textsubscript{2} populations.

<table>
<thead>
<tr>
<th>F\textsubscript{2} Population</th>
<th>Number of plants</th>
<th>The minimum value of the root shape index</th>
<th>The maximum value of the root shape index</th>
<th>No. of plants in the extreme pools</th>
<th>Mean value of the extremely small pool</th>
<th>Mean value of the extremely large pool</th>
</tr>
</thead>
<tbody>
<tr>
<td>1902</td>
<td>432</td>
<td>0.608</td>
<td>4.109</td>
<td>35</td>
<td>1.105</td>
<td>3.253</td>
</tr>
<tr>
<td>1908</td>
<td>397</td>
<td>0.847</td>
<td>3.643</td>
<td>40</td>
<td>1.142</td>
<td>2.880</td>
</tr>
<tr>
<td>1909</td>
<td>453</td>
<td>0.69</td>
<td>4.468</td>
<td>45</td>
<td>0.893</td>
<td>2.957</td>
</tr>
</tbody>
</table>

Correlation among the root shape characteristics in radish

The correlation analysis was conducted based on the phenotypic data of the 1282 F\textsubscript{2} individuals. The results showed that root length had a high positive correlation with the root shape index, and root diameter was negatively correlated with the root shape index (Table S1). In the three F\textsubscript{2} populations, the correlation between root length and the root shape index was 0.667, 0.800, and 0.845, and the correlation between root diameter and the root shape index was -0.294, -0.438, and -0.299. There was no significant correlation between root length and root diameter. Thereby, the root shape was predominantly determined by root length.

Sequencing of the parents and mixed pools

Approximately 35, 40 and 45 individuals with extreme phenotypes were selected for mixed pool sequencing for populations 1902, 1908 and 1909, respectively. The four parents and six mixed pools of three F\textsubscript{2} populations of radish were subjected to Illumina sequencing. A total of 152.11 Gb data were generated, with
the average Q30 reaching 93.12% and the GC content ranging from 37.26% to 38.00% (Table 3). The average map-ping efficiency between the samples and the reference genome was 96.23%. The map-ping rate was mostly around 96%, whereas it was only 92.54% between the leaf radish CZ and the reference genome, which was the lowest among all the samples. The average sequencing depth of the parents was 16.40 ×, and the average depth of the extreme pools was 23.00 ×. The genome coverage was 87.00% (at least one base coverage). The quality of the sequencing data was appropriate to meet the requirements of QTL-seq analysis.

**Table 3** Statistics of the sequencing data for the parents and extreme pools.

<table>
<thead>
<tr>
<th>Name</th>
<th>Clean Reads</th>
<th>Clean Base</th>
<th>Q30(%)</th>
<th>GC(%)</th>
<th>Mapped(%)</th>
<th>Cov_ratio_1X(%)</th>
<th>Average depth</th>
</tr>
</thead>
<tbody>
<tr>
<td>CZ</td>
<td>40,770,993</td>
<td>12,203,297,052</td>
<td>92.81</td>
<td>38.00</td>
<td>92.54</td>
<td>84.62</td>
<td>16</td>
</tr>
<tr>
<td>LLYH</td>
<td>38,237,712</td>
<td>11,441,633,036</td>
<td>93.57</td>
<td>37.40</td>
<td>95.91</td>
<td>82.79</td>
<td>17</td>
</tr>
<tr>
<td>BY</td>
<td>35,629,299</td>
<td>10,664,593,300</td>
<td>92.31</td>
<td>37.54</td>
<td>96.86</td>
<td>83.06</td>
<td>16</td>
</tr>
<tr>
<td>CLA</td>
<td>34,695,079</td>
<td>10,383,258,026</td>
<td>92.89</td>
<td>37.45</td>
<td>96.64</td>
<td>82.82</td>
<td>15</td>
</tr>
<tr>
<td>R05</td>
<td>37,984,421</td>
<td>11,363,953,126</td>
<td>92.95</td>
<td>37.40</td>
<td>96.73</td>
<td>81.95</td>
<td>18</td>
</tr>
<tr>
<td>S-pool-1</td>
<td>53,006,850</td>
<td>15,872,724,858</td>
<td>92.36</td>
<td>37.43</td>
<td>96.67</td>
<td>90.20</td>
<td>22</td>
</tr>
<tr>
<td>L-pool-1</td>
<td>52,964,015</td>
<td>15,850,467,634</td>
<td>93.92</td>
<td>37.34</td>
<td>96.66</td>
<td>90.25</td>
<td>23</td>
</tr>
<tr>
<td>S-pool-2</td>
<td>56,828,841</td>
<td>17,010,808,844</td>
<td>93.20</td>
<td>37.26</td>
<td>96.83</td>
<td>90.23</td>
<td>24</td>
</tr>
<tr>
<td>L-pool-2</td>
<td>53,796,882</td>
<td>16,102,268,428</td>
<td>93.74</td>
<td>37.32</td>
<td>96.84</td>
<td>90.26</td>
<td>23</td>
</tr>
<tr>
<td>S-pool-3</td>
<td>52,592,836</td>
<td>15,736,253,794</td>
<td>93.85</td>
<td>37.30</td>
<td>96.58</td>
<td>90.10</td>
<td>23</td>
</tr>
<tr>
<td>L-pool-3</td>
<td>51,721,719</td>
<td>15,485,509,054</td>
<td>92.75</td>
<td>37.36</td>
<td>96.28</td>
<td>90.71</td>
<td>22</td>
</tr>
</tbody>
</table>

**Detection of QTLs**

A total of seven QTLs associated with the root shape index were detected using QTL-seq, which were distributed on four radish chromosomes: chromosome 1 (R1; rs1.1), chromosome 2 (R2; rs2.1), chromosome 4 (R4; rs4.1 and rs4.2), chromosome 5 (R5; rs5.1) and chromosome 7 (R7; rs7.1 and rs7.2) chromosomes (Fig. 3, Table 4). rs4.1 was detected in the 1902 population and located within the 40.51-42.59 Mb region of...
chromosome R4, while the rs4.2 (42.71-45.81 Mb) locus was detected in the 1909 population and was very close to rs4.1. Nonetheless, there was no overlapping between the two QTLs on chromosome 4. rs7.1 was detected in the 1908 population (9.92-14.81 Mb), and rs7.2 was detected in the 1909 (13.79-15.35 Mb) population, with an overlap of 1.02 Mb (13.79-14.81 Mb). However, the two populations did not share common parents. Although the 1902 and 1908 populations shared a common parent (CLA) we did not detect the same QTLs. rs1.1 (13.06-16.37 Mb) and rs5.1 (27.54-28.60 Mb) were detected only in the 1902 population, while rs2.1 (32.07-33.43 Mb) was detected only in the 1909 population. The number of SNPs ranged from 2106-7471 SNPs in the seven QTL regions, among which 94368 nonsynonymous SNPs were found. Detailed information on the SNPs and annotation information for the genes with nonsynonymous SNPs are listed in Table S2 and Table S3. Further work is still needed to obtain candidate genes that regulate root shape from these SNPs, such as fine mapping or comprehensive omics analyses, to screen and obtain the candidate genes and carry out functional verification.

Table 4 Summary of QTLs detected for the root shape index with QTL-seq.

<table>
<thead>
<tr>
<th>Population</th>
<th>QTL</th>
<th>Chr.</th>
<th>Start</th>
<th>End</th>
<th>Interval (Mb)</th>
<th>No. of SNPs in the interval</th>
<th>No. of genes with nonsynonymous mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1902</td>
<td>rs1.1</td>
<td>R1</td>
<td>13.06</td>
<td>16.37</td>
<td>3.31</td>
<td>4753</td>
<td>222</td>
</tr>
<tr>
<td></td>
<td>rs4.1</td>
<td>R4</td>
<td>40.51</td>
<td>42.59</td>
<td>2.08</td>
<td>5597</td>
<td>215</td>
</tr>
<tr>
<td></td>
<td>rs5.1</td>
<td>R5</td>
<td>27.54</td>
<td>28.60</td>
<td>1.06</td>
<td>2279</td>
<td>94</td>
</tr>
<tr>
<td>1908</td>
<td>rs7.1</td>
<td>R7</td>
<td>9.92</td>
<td>14.81</td>
<td>4.89</td>
<td>7471</td>
<td>368</td>
</tr>
<tr>
<td>1909</td>
<td>rs2.1</td>
<td>R2</td>
<td>32.07</td>
<td>33.43</td>
<td>1.36</td>
<td>5514</td>
<td>132</td>
</tr>
<tr>
<td></td>
<td>rs4.2</td>
<td>R4</td>
<td>42.71</td>
<td>45.81</td>
<td>2.10</td>
<td>2106</td>
<td>122</td>
</tr>
<tr>
<td></td>
<td>rs7.2</td>
<td>R7</td>
<td>13.79</td>
<td>15.35</td>
<td>1.56</td>
<td>4954</td>
<td>137</td>
</tr>
</tbody>
</table>

Discussion

Root formation is one of the most important traits for the radish cultivars especially in (Asia Hashida T et al. 2013), which are consumed fresh or cooked. Root shape is an important trait that determines commercial quality in radish. The root shape of radish is not only regulated by multiple QTL genes, but also affected by many complex factors, such as the environmental and physiological conditions. However, the mapping/fine mapping of QTLs or related genes is difficult due to the influence of many environmental factors. Studies have shown that external factors such as light, temperature, moisture, mineral nutrition, carbon dioxide and soil conditions could affect the length and diameter of fleshy radish roots, thus affecting the root shape index (Mckenna A et al. 2010; Cingolani P 2012). At the same time, the formation process of radish roots is also influenced by hormones, such as auxin, cyto-kinin, gibberellin and ethylene, which play roles in determining root length and diameter (Altschul S 2000; Kanehisa M et al. 2004).

In this study, five radish parents showed great differences in their root shape indexes and were used to construct three separate F₂ populations (Fig. 1). All the materials were planted in sandy land with loose soil,
and the water and fertilizer regimes were kept consistent and balanced during the growth process to minimize the influence of external factors on the development of fleshy roots and to ensure the accuracy of the phenotypic data. The growth periods of the parental radishes showed comparatively large differences. The normal growth cycle differs by nearly 45 d, so the growth cycle of each radish in the F_2 population is also different. Since radish roots grow underground, it is difficult to measure the phenotypic data directly, and radishes must be harvested to measure traits. This increases the statistical error of the phenotypic data and the difficulty of gene mining, also reduces the accuracy of QTL mapping. However, the interaction between genetic effects and growth stages did not appreciably affect the inheritance of root shape among growth stages, and root shape can be efficiently selected at an early stage (Hiroyoshi I et al. 2004). In this study, centralized and unified harvesting was adopted. Phenotypic data on the fleshy root length and diameter were measured on the 45th day of the growth period. The root shape index in the three F_2 populations ranged from 0.82 to 5.52.

Previous studies have focused on the transcriptome, miRNA, and proteome of radish roots at different developmental stages. 11 genes were found significantly differentiated between two radishes with different fleshy root shapes using suppression subtractive hybridization (Zaki HEM et al. 2010). Mitsui et al. (Mitsui Y et al. 2015) conducted detailed biological observations and transcriptome analyses of different developmental stages of roots. The results showed that genes related to carbohydrate metabolism were active in the developing roots, especially in the tissues undergoing cell proliferation. Moreover, the sucrose synthase (SUS1) gene was also related to the development of roots (Mitsui Y et al. 2015). Two sucrose metabolism genes (RsSuSy1 and RsSPS1) and the RsCLE41 and RsSAUR genes were cloned and analyzed, which are related to root formation in radish (Altschul S 1997). A total of 191 target genes with differentially expressed miRNAs during the enlargement of fleshy roots were predicted, and 114 miRNA-mRNA pairs were identified through the association analysis of DEGs and miRNAs (Altschul S 1997). Muvva et al. identified 48 conserved miRNAs in radish and predicted 16 potential genes involving radish root shape (Kanehisa M et al. 2004). The differentially expressed genes found in the above studies useful for the mining of candidate genes in the QTL region and verify whether the candidate genes are related to the shape of the radish root. The root shape index and even the expansion mechanism of radish fleshy roots can be systematically analyzed by the combined methods of genomics, transcriptomics, proteomics and metabolomics. However, those studies were referred to different versions of radish genomes, and the differences between the genome versions also make it difficult to summarize and analyze the candidate gene sequences. Thus, although many different genes were found in previous studies, it was difficult to find and summarize the related sequence information of these genes.

At present, few studies have been conducted on the QTL mapping of radish root shape index traits. A radish genetic map was constructed and used for QTL analysis using a segregated F_2 population and 198 markers, including 169 AFLPs, 28 Brassica SSRs and one SLG-CAPS (Masato Tsuro et al. 2008). Three QTLs were detected for the root shape index of radish, which were located on three linkage groups (LG3, 8 and 9), explaining 42.4% of the phenotypic variation. Moreover, two QTLs controlling root diameter were also detected on LG4 and LG8, among which the QTL on LG 8 also affected root shape via diameter (Masato Tsuro et al. 2008). Hashida et al. (Hashida T et al. 2013) constructed a genetic map composed of 322 markers with a recombinant inbred line (RIL) population and assigned the linkage groups to radish chromosomes. Seven
QTLs were associated with fleshy root weight with two-year trait data, which were located on two radish chromosomes, chromosome H (R7) and chromosome I (R2). Although previous studies have been reported on root shape index mapping using genetic maps (Masato Tsuro et al. 2008), it is difficult to compare them with each other or the results from the present study due to the insufficient accuracy of the genetic maps. In this study, seven QTLs for the root shape index were detected using three F$_2$ populations by QTL-seq methods, distributing on four radish chromosomes, two QTLs on R4 and R7, respectively and one QTL for each of the R1, R2, and R5 (Fig. 3, Table 4). Among them, the rs7.1 in the 1908 population and rs7.2 in the 1909 population had an overlapping region of 1.02 Mb, despite that the two populations did not share common parents. The genes related to nonsynonymous SNPs were annotated and listed in Table S2 and Table S3. Nonetheless, more QTL mapping studies on the radish root shape index are needed to find consistent QTLs in combination with special populations. Furthermore, it is still needed to obtain candidate genes that regulate root shape from these SNPs and carry out functional verification. The present study laid a foundation for subsequent fine mapping and gene mining associated with radish root shape characteristics.

**Conclusions**

In this study, three segregating F$_2$ populations derived from independent crosses were developed and evaluated for root shape index traits. Combined with the latest radish reference genome, seven QTLs related to the root shape index were obtained by QTL-seq analysis. The number of SNPs within the seven QTL regions was calculated and the genes associated with the nonsynonymous SNPs were annotated. These results laid a foundation for the development of molecular markers linked to root shape index traits and provided an important reference for fine mapping the genes controlling root shape.

**Declarations**

**Ethics approval and consent to participate** This article does not contain any studies with human participants or animals performed by any of the authors.

**Consent for publication** Not applicable.

**Availability of data and materials** All the data and plant material are available with the corresponding author.

**Competing interests** The authors declare that they have no competing interests.

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**Authors’ contributions** Tianhua Hu and Qingzhen Wei conceived and designed the experiments, performed the experiments, analyzed the data, and wrote the paper. Jinglei Wang participated in part of the data analysis. Wuhong Wang, Haifiao Hu and Yaqin Yan were involved in the cultivation and trait statistics of radish materials in the field. Chonglai Bao conceived and designed the experiments, authored or reviewed drafts of the paper, approved the final draft.
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**Figures**
Figure 1

Roots of the parents at their corresponding complete growth periods and the roots of individuals with extreme phenotypes at 45 d of growth. (a) Parents of the CZ×CLA cross and extreme individuals from the 1902 population. (b) Parents of the R05×CLA cross and extreme individuals from the 1908 population. (c) Parents from the LLYH×BY cross and extreme individuals from the 1909 population.
Figure 2

Frequency distribution of the root shape index in three radish F$_2$ populations.
Figure 3

The $\triangle$(SNP-index) distribution of the QTLs for the root shape index detected by QTL-seq. (a) The $\triangle$(SNP-index) distribution of the 1902 population. (b) The $\triangle$(SNP-index) distribution of the 1908 population. (c) The $\triangle$(SNP-index) distribution of the 1909 population.

Supplementary Files

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