Introduction

Sunflower (Helianthus annuus L.) is one of the most important vegetable oil sources worldwide. Cultivated sunflower is a diploid species (2n = 2x = 34) with a large genome size of 3.6 Gb (Badouin et al. 2017). Between 2007 and 2016, approximately 24.800 million hectares of sunflower crop were cultivated globally per annum, of which approximately 0.932 million hectares were cultivated in China (Food and Agricultural Organization of the United Nations Statistical Databases 2017; http://www.fao.org/faostat/en/#home).

Genetic maps are a key resource for understanding genome organization and evolutionary relationships (Wang et al. 2011) and are particularly valuable tools for QTL mapping and marker-assisted selection (MAS). Because many important agronomic traits of sunflower are controlled by numerous loci, quantitative trait locus (QTL) analysis and MAS can shorten breeding duration and accelerate breeding efficacy of new sunflower varieties. To date, several sunflower genetic linkage maps incorporating different molecular markers have been constructed based on restriction fragment length polymorphisms (RFLPs) (Berry et al. 1995, Gentzbittel et al. 1995, Jan et al. 1998), random amplification of polymorphic DNA (RAPD) (Rieseberg et al. 1993), target region amplification polymorphisms (TRAPs) (Bing et al. 2008, Maleki et al. 2014), amplified fragment length polymorphisms (AFLPs) (Kusterer et al. 2004, Maleki et al. 2014), simple sequence repeats (SSRs) (Talia et al. 2010, Tang et al. 2002, Yu et al. 2003), single nucleotide polymorphisms (SNPs) (Hulke et al. 2015), and sequence-related amplification polymorphisms (SRAPs) (Lyu et al. 2017). Until recently, most such maps were based on low-throughput marker technologies, which resulted in genetic maps of limited density. These older maps have since been eclipsed by higher density maps generated using new high-throughput genotyping technologies such as restriction site-associated DNA (RAD) tag sequencing (Talukder et al. 2014). Nevertheless, with the recent release of the sunflower genome (Badouin et al. 2017) and the development of genome...
sequencing technologies, soon SNPs will be the predominant markers used for construction of genetic maps and for marker-assisted breeding. Indeed, as single nucleotide DNA sequence variants, SNPs are currently markers of choice for high-density genetic map construction due to their abundance, uniform genome distribution, and low-cost detection (Ganal et al. 2009).

Plant height, head diameter, and stem diameter are important agronomic traits in sunflower, with known effects on yield and lodging, while fatty acid composition influences the quality and stability of sunflower oil. One advantage of sunflower oil, which is high in oleic acid, is its high degree of oxidative stability that is desirable for cooking, refining, and storage uses, as compared to oils low in oleic acid (Fuller et al. 1967). To date, most reported molecular markers related to sunflower plant height, head diameter, stem diameter, and oleic acid content have been nonfunctional markers and even false positive QTLs, impeding progress in marker-assisted selection (MAS). The construction of a high-density SNP map of sunflower will improve the efficiency and accuracy of localization of important sunflower QTLs to benefit future in-depth discovery and development of functional markers and MAS in sunflower.

SLAF-seq is a new technology that can be used to rapidly develop SNP markers after construction of a SLAF-seq library (Sun et al. 2013). Due to its advantages of high-throughput, high accuracy, low cost, and short cycle times, SLAF-seq technology has been successfully applied to construct high-density maps of watermelon (Shang et al. 2016), flue-cured tobacco (Gong et al. 2016), pear (Wang et al. 2017b), soybean (Li et al. 2017), cucumber (Xu et al. 2015), rice (Peng et al. 2016), sorghum (Li et al. 2017), and grape (Wang et al. 2017a).

In this study, to achieve rapid mass discovery of SNP markers and high-density genetic mapping of sunflower, two elite sunflower (Helianthus annuus L.) cultivars ‘86-1’ and ‘L-1-OL-1’ were used as female and male parents, respectively. Next, SLAF-seq technology was used to develop SLAF markers using genetic material from both the F2 hybrid population and parents to construct a high-density linkage map. Based on this high-density genetic map, QTLs associated with oleic acid content and three agronomic traits (plant height, head diameter, and stem diameter) were identified. These results will likely facilitate future development of functional markers and the application of MAS to sunflower breeding.

Materials and Methods

Plant materials

An F2 mapping population derived from a cross of sunflower inbred lines ‘L-1-OL-1’ (male parent, bred in Serbia) and ‘86-1’ (female parent, bred in China) was used to construct a linkage map. The seed of ‘L-1-OL-1’ and ‘86-1’ differ significantly in oleic acid content, with ‘L-1-OL-1’ exhibiting higher oleic acid content (87.65%) than ‘86-1’ (18.61%). Both parents and their 84 F2 progeny were planted in the experimental field of Industrial Crops Institute, Heilongjiang Academy of Agricultural Sciences in Harbin, China.

DNA extraction

Young healthy leaves from F2 individuals and parents were sampled and genomic DNA was extracted using a DNasecure Plant Kit (TIANGEN Biotech, Beijing, China).

SLAF library construction and high-throughput sequencing

SLAF-seq was used to genotype 84 F2 individuals and their parents, as previously described with minor modifications (Sun et al. 2013). The digestion sites and the lengths and length distribution of resulting fragments were predicted using the reference genome. Three criteria were considered: i) The number of SLAFs must be suitable for the specific needs of the research project. ii) The SLAFs must be evenly distributed throughout the sequences to be examined. iii) Repeated SLAFs must be avoided (Sun et al. 2013). The HaeIII restriction enzyme was finally chosen for use in achieving complete digestion of genomic DNA of the mapping (F2 population) (New England Biolabs, USA) followed by addition of a single-nucleotide (A) overhang to each digested fragment using Klenow Fragment (3′→5′exo–) (NEB) and dATP at 37°C. Duplex tag-labeled sequencing adapters (PAGE-purified, Life Technologies, USA) were ligated to the A-tailed fragments using T4 DNA ligase.

Polymerase chain reaction (PCR) was performed using diluted restriction enzyme-digested and ligated DNA samples, dNTP, Q5® High-Fidelity DNA Polymerase (NEB), and PCR primers (Forward primer: 5′-AATGATACGGCGACGTGCTCTTCTCATAGATGCCAGACGCGTAA-3′, reverse primer: 5′-CAAGCGAAGACGGCATCGT-3′) (PAGE purified, Life Technologies). PCR products were then purified using Agencourt AMPure XP beads (Beckman Coulter, High Wycombe, UK) and pooled. Pooled samples were separated by 2% agarose gel electrophoresis. Fragments ranging from 314 to 444 bp in size (including indexes and adaptors) were excised and purified using a QIAquick Gel Extraction Kit (Qiagen, Hilden, Germany). Gel-purified products were then quantified and appropriately diluted. Paired-end sequencing (of each end of 125 bp) was performed using an Illumina HiSeq 2500 system (Illumina, Inc., San Diego, CA, USA) according to the manufacturer’s recommendations.

Sequence data grouping and genotyping

SLAF marker identification and genotyping were performed using procedures previously described (Sun et al. 2013). The genome of Helianthus annuus L. (Badouin et al. 2017) (https://www.ncbi.nlm.nih.gov/genome/?term=Helianthus%3Ananus%5Borgn%5D) was used as a reference. Briefly, low-quality reads (quality score < 30) were filtered out and remaining raw reads were assigned to each progeny according to duplex barcode sequences. After the barcodes and terminal 5-bp sequences were trimmed from each
high-quality read, clean reads were clustered together according to their sequence identities. Sequences mapping to the same locus with over 90% identity were defined as one SLAF locus (Zhang et al. 2015). Single nucleotide polymorphic (SNP) loci for each SLAF locus were then assigned to each parent and SLAFs with more than 3 SNPs between parents were filtered out first. Alleles of each SLAF locus were then defined from parental reads with sequence depth >10-fold. For diploid species, one SLAF locus can contain a maximum of four genotypes; therefore, SLAF loci with more than four alleles were defined as repetitive SLAFs and were discarded. Only SLAFs with two to four alleles were defined here as polymorphic and considered as potential markers.

All polymorphic SLAFs loci were genotyped based on their consistency within both parental and offspring SNP loci. Marker codes of polymorphic SLAFs were next analyzed according to the population type F2, which consisted of one segregation type (aa × bb). Genotype scoring was then performed using a Bayesian approach to further ensure genotype quality (Sun et al. 2013). Subsequently, high-quality SLAF markers for genetic mapping were retained using the following criteria. First, average sequence depths should be >10-fold in the parents. Second, markers with more than 30% missing data were filtered out and discarded. Third, the chi-square test was performed to examine the severity of segregation distortion. Markers with significant segregation distortion (p < 0.01) were excluded from map construction.

High-density genetic map construction

Marker loci were partitioned primarily into linkage groups (LGs) using modified logarithm of odds (MLOD) score values of >5 as a cutoff value. To ensure efficient construction of the high-density and high-quality map, the HighMap strategy was utilized to arrange SLAF markers in a specific order and correct genotyping errors within LGs (Liu et al. 2014). Specifically, the genetic map was constructed according to the maximum likelihood method (Van Ooijen 2011) and genotyping errors were corrected using the SMOOTH algorithm (Van Os et al. 2005). A k-nearest neighbor algorithm was applied to impute missing genotypes (Huang et al. 2011). Finally, genetic map distances were estimated using the Kosambi mapping function (Kosambi 1943).

Phenotypic data collection

The oleic acid content values of seeds were measured at the Oil Crops Research Institute, China Academy of Agricultural Sciences using gas chromatography (model Agilent 7890A, USA). Plant height (PH), head diameter (HD), and stem diameter (SD) were measured during the full flowering period before harvest. The stem diameter was measured through the center of each stem.

QTL analysis using a high-density genetic map

QTLs that mapped to oleic acid content and the three other aforementioned agronomic traits were identified using the composite interval mapping (CIM) method application within R/qtl software. The threshold of LOD scores for evaluating the statistical significance of QTL effects was determined using 1,000 permutations. Based on these permutations, a LOD score of 2.0 was used as the minimum cutoff for declaring the presence of a QTL within a particular genomic region.

Availability of data

The dataset supporting the conclusions of this article has been available in The European Nucleotide Archive as accession number PRJEB25920 (https://www.ebi.ac.uk/ena/submit/sra/).

Results

Analysis of SLAF sequencing data and SLAF markers

A total of 105.60 Gb of raw reads consisting of 530.50 Mb paired-end reads were obtained using high-throughput sequencing of SLAF libraries. Among those reads, the average percentage of Q30 bases (bases with a quality score of 30, indicating a 1% chance of an error and thus a 99% confidence level) was 90.84% and the GC content was 44.36% (Supplemental Table 1). There were 4,505,523 reads from the female parent and 4,839,674 reads from the male parent and an average number of reads for the F2 population of 2,488,845. SLAF numbers obtained from the male and female parents were 266,226 and 252,824, respectively (Table 1). The average sequencing depth was 18.18-fold for the male parent and 17.82-fold for the female parent. In the F2 population, the average number of SLAF markers was 258,665 and the average coverage was 9.62-fold (Fig. 1). After read clustering was completed, a total of 343,197 SLAFs were detected.

Among the 343,197 SLAFs obtained, 39,589 were polymorphic, with a polymorphism rate of 11.54%, while the remaining 303,045 were non-polymorphic and 563 were repetitive. After filtering out SLAF markers lacking parental information, 27,261 polymorphic markers were successfully classified into eight segregation patterns (Fig. 2). The F2 population was obtained by selfing of the F1 progeny obtained from a cross between two parents with homozygous genotype of aa or bb. Consequently, only F2 plants with the aa × bb segregation pattern were used for further analysis, yielding a total of 19,282 markers that fell within this segregation pattern type. Among these 19,282 markers, 6,136

Table 1. Summary of marker depths

<table>
<thead>
<tr>
<th>Samples</th>
<th>Marker numbers</th>
<th>Total depth</th>
<th>Average depth</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-1-OL-1 (P)</td>
<td>266,226</td>
<td>4,839,674</td>
<td>18.18</td>
</tr>
<tr>
<td>86-1 (M)</td>
<td>252,824</td>
<td>4,505,523</td>
<td>17.82</td>
</tr>
<tr>
<td>Offspring</td>
<td>258,665</td>
<td>2,488,845</td>
<td>9.62</td>
</tr>
</tbody>
</table>
Genetic map and QTL for oleic acid and agronomic traits

markers showing extremely significant (p < 0.01) segregation distortion.

Basic characteristics of the genetic map

In total, 6,136 markers were grouped into 17 linkage groups (LGs). The average integrity of mapped markers was 99.79%, indicating an acceptably high genetic map quality (Supplemental Tables 2–4). The total genetic length of the map was 2221.86 cM, with an average distance between adjacent markers of 0.36 cM (Fig. 3). All LGs are shown in Table 2. On average, each LG contained 361 markers that spanned an average of 130.70 cM. The lengths of LGs ranged from 102.72 cM (LG16) to 156.86 cM (LG11). LG11, the longest LG, had a genetic length of 156.86 cM and contained 239 markers with an average distance between markers of 0.66 cM. LG16, the shortest LG, had a genetic length of 156.86 cM and contained 239 markers with an average distance between markers of 0.66 cM. LG16, the shortest LG, had a genetic length of 102.72 cM and contained 597 markers, with an average inter-marker distance of 0.17 cM. LG17 contained the maximum number of markers (801), whereas LG4 possessed the minimum marker number (78). The “Gap ≤5” value ranged from 92.49% to 100%, with a total of 44 gaps of length >5 cM. Of these, a total of 40 gaps were 5 to 10 cM in length and four gaps had lengths >10 cM. The largest gap on this map, 16.40 cM in length, was located within LG4. Most gaps (13) of lengths greater than 5 cM were present within LG1, while LG6, LG12, and LG17 contained no gap with length greater than 5 cM (Table 2, Supplemental Table 4). A total of 11,980 SNP markers were used for construction of the final genetic map after discarding the remainder, which were homozygous between parents. Ultimately, a sequence depth for the parents of greater than 10-fold was achieved, with over 70% integrity of SLAF tags and exclusion from the final map of...
ic maps were of high quality. Heat maps were generated using pair-wise recombination values for the 6,136 mapped SLAFs (Supplemental Data 2), since heat maps may reflect the recombination relationship between markers from each LG and are useful for finding potential ordering errors. Notably, heat maps developed here indicate that the ordering of SLAF markers within most LGs was correct.

Segregation distortion of markers on the map
Only 25 (0.41%) of the 6,136 markers on the genetic map exhibited significant segregation distortion (p < 0.01). Notably, markers exhibiting segregation distortion were only distributed within three LGs, LG4 (17.95%), LG9 (4.18%), and LG11 (0.42%). No significant correlation was found between the distribution of mapped markers and distorted markers. For example, LG17 contained the maximum number of markers (801 markers) and covered 141.02 cM, but did not contain any distorted markers. For comparison, LG4, which possessed the minimum SLAF marker number (78 markers) and covered 112.03 cM, contained 14 distorted markers.

Phenotypic analysis of oleic acid content and 3 agronomic traits
Phenotypic data for the two parents and F2 families are shown in Table 4. Of these, plant height shows the minimum coefficient of variance (CV) of 9.78%, while oleic acid content exhibits the maximum CV of 39.43%. The frequency distributions of four phenotypic traits were also analyzed and almost all exhibited continuous and normal distributions, with the exception of oleic acid content (Fig. 4, Table 4).

QTL analysis using the high-density genetic map
Based on the high-density genetic map, QTLs for plant
height, head diameter, stem diameter, and oleic acid content were identified. As a result, QTLs with LOD scores above 2.0 were considered to be effective QTLs (Supplemental Fig. 1). According to the threshold, two QTLs (PH_1 and PH_2) were detected for plant height and accounted for 10.31% and 12.28% of phenotypic variance, respectively. Two QTLs (HD_1 and HD_2) were detected for head diameter and accounted for 5.63% and 5.49% of phenotypic variance, respectively. For stem diameter, one QTL (SD_1) accounted for 15.65% of the phenotypic variance of that trait. For oleic acid, three QTLs (OAC_1, OAC_2, and OAC_3) accounted for 12.05%, 5.81%, and 5.18% of phenotypic variance, respectively (Table 5). These QTLs mapped to LG5, LG6, LG7, LG9, LG16, and LG17 (Fig. 5), with LOD scores ranging from 2.15 to 2.78 (Table 5).

Table 4. The characteristics of the phenotypic traits in sunflower F2 mapping population

<table>
<thead>
<tr>
<th>Traits</th>
<th>L-1-OL-1 (%)</th>
<th>86-1 (%)</th>
<th>Mean (%)</th>
<th>Min (%)</th>
<th>Max (%)</th>
<th>SD</th>
<th>Coefficient of variance</th>
<th>Kurtosis</th>
<th>Skewness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oleic acid</td>
<td>87.65</td>
<td>18.61</td>
<td>57.19</td>
<td>17.32</td>
<td>89.10</td>
<td></td>
<td>22.55</td>
<td>0.3943</td>
<td>–1.22</td>
</tr>
<tr>
<td>Plant height</td>
<td>125.2</td>
<td>150.1</td>
<td>166.93</td>
<td>128.1</td>
<td>225.3</td>
<td></td>
<td>16.33</td>
<td>0.0978</td>
<td>1.26</td>
</tr>
<tr>
<td>Head diameter</td>
<td>23.1</td>
<td>18.8</td>
<td>22.33</td>
<td>11.5</td>
<td>32.2</td>
<td></td>
<td>4.23</td>
<td>0.1894</td>
<td>–0.10</td>
</tr>
<tr>
<td>Stem diameter</td>
<td>2.19</td>
<td>2.41</td>
<td>2.49</td>
<td>1.71</td>
<td>3.63</td>
<td></td>
<td>0.41</td>
<td>0.1647</td>
<td>0.38</td>
</tr>
</tbody>
</table>

Table 5. Quantitative trait loci (QTL) analysis of sunflower the target traits

<table>
<thead>
<tr>
<th>Trait</th>
<th>QTL</th>
<th>LG ID</th>
<th>Marker number</th>
<th>Interval (cM)</th>
<th>LOD</th>
<th>ADD</th>
<th>PVE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant height</td>
<td>PH_1</td>
<td>7</td>
<td>2</td>
<td>84.223–84.223</td>
<td>2.52</td>
<td>6.69</td>
<td>10.31</td>
</tr>
<tr>
<td></td>
<td>PH_2</td>
<td>17</td>
<td>5</td>
<td>40.511–40.511</td>
<td>2.50</td>
<td>7.84</td>
<td>12.28</td>
</tr>
<tr>
<td>Head diameter</td>
<td>HD_1</td>
<td>5</td>
<td>6</td>
<td>46.639–48.708</td>
<td>2.78</td>
<td>1.14</td>
<td>5.63</td>
</tr>
<tr>
<td></td>
<td>HD_2</td>
<td>9</td>
<td>2</td>
<td>91.156–92.943</td>
<td>2.15</td>
<td>1.28</td>
<td>5.49</td>
</tr>
<tr>
<td>Stem diameter</td>
<td>SD_1</td>
<td>16</td>
<td>11</td>
<td>7.266–7.266</td>
<td>2.61</td>
<td>0.10</td>
<td>15.65</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>OAC_1</td>
<td>9</td>
<td>3</td>
<td>125.078–131.277</td>
<td>2.50</td>
<td>–8.41</td>
<td>5.81</td>
</tr>
<tr>
<td></td>
<td>OAC_2</td>
<td>6</td>
<td>5</td>
<td>95.168–99.576</td>
<td>2.50</td>
<td>–8.41</td>
<td>5.81</td>
</tr>
<tr>
<td></td>
<td>OAC_3</td>
<td>6</td>
<td>3</td>
<td>109.108–109.108</td>
<td>2.23</td>
<td>–7.20</td>
<td>5.18</td>
</tr>
</tbody>
</table>
Of these, 39,589 were polymorphic, with a polymorphism rate of 11.54%. After a series of screenings, 6,136 markers containing 11,980 SNP loci were mapped successfully onto the high-density genetic map. The number of polymorphic markers within each chromosome ranged from 78 to 801, with the final set of SLAF markers obtained covering all sunflower LGs. Furthermore, marker accuracy and integrity determined from haplotype and heat maps were high (99.79%), indicating that marker quality was suitable for construction of a genetic map. In conclusion, the number of sunflower polymorphic markers obtained using SLAF-seq technology was higher than obtained using previous methods, further demonstrating that SLAF-seq is an efficient method for obtaining abundant polymorphic markers to rapidly construct high-density genetic maps.

Several genetic maps have been previously reported for sunflower and most were generated using markers obtained using SSR alone or in combination with other markers. Within these maps, the number of mapped markers was relatively small, with relatively large average distances separating adjacent markers. One sunflower genetic map included 459 SSR markers based on 94 F7 recombinant inbred lines (RILs) that mapped to 17 linkage groups. The map length was 1,368.3 cM, with a mean density of 3.1 cM/locus. Meanwhile, another sunflower genetic map incorporating 202 AFLP and 19 SSR markers based on

**Discussion**

Genetic maps play an important role in the identification of functional genes and QTLs of interest. Moreover, these maps are a highly valuable platform for use in comparative analysis of genome structure and for MAS. However, only a few hundred markers are included in most current genetic maps, due to ineffective discovery technologies and high genotyping costs. The fusion of powerful sequencing technologies with genotyping approaches, including restriction site-associated DNA sequencing (RAD-seq), genotyping-by-sequencing (GBS), and SLAF-seq, allow millions of SNPs to be identified within plant genomes. SLAF-seq has a major advantage in that it allows researchers to first design the experimental system using bioinformatics analysis to screen for fragments of a chosen specific length from a constructed SLAF-seq library. Consequently, SLAF fragment length selection is not plagued by random interruptions and thus achieves better repeatability over RAD-seq and GBS. Moreover, large amounts of sequence information can be generated and whole genome density distributions can be handled using SLAF-seq (Qi et al. 2014) to generate markers with higher density, better consistency, greater effectiveness, and at lower cost than using traditional methods. In this study, 105.60 Gb of raw data containing 530.50 M reads were generated using SLAF-seq, for a total yield of 343,197 SLAF markers. Of these, 39,589 were polymorphic, with a polymorphism rate of 11.54%. After a series of screenings, 6,136 markers containing 11,980 SNP loci were mapped successfully onto the high-density genetic map. The number of polymorphic markers within each chromosome ranged from 78 to 801, with the final set of SLAF markers obtained covering all sunflower LGs. Furthermore, marker accuracy and integrity determined from haplotype and heat maps were high (99.79%), indicating that marker quality was suitable for construction of a genetic map. In conclusion, the number of sunflower polymorphic markers obtained using SLAF-seq technology was higher than obtained using previous methods, further demonstrating that SLAF-seq is an efficient method for obtaining abundant polymorphic markers to rapidly construct high-density genetic maps.
In this study, an F2 mapping population that was employed for marker-assisted selection or chromosomal rearrangements (Menz et al. 2002). Yet another sunflower genetic map was reported that incorporated 547 markers (231 SSR, 9 EST-SSR, 3 InDels, and 304 AFLPs based on 94 RILs) that mapped to 17 linkage groups (LGs); the map had an overall length of 1,942.3 cM and mean density of one marker per 3.6 cM (Talia et al. 2010). Another sunflower genetic map, containing 88 AFLP and 44 SSR markers derived from 101 F2 individuals, contained 17 LGs and possessed an overall length of 1,490 cM and mean density of one marker per 12.44 cM (Maleki et al. 2014). Recently, the availability of high-throughput genotyping technologies has made it possible to construct even higher density genetic maps. One map incorporated 5,019 SNP markers obtained using RAD tag sequencing of 118 publicly available SSR markers; this map integrated SNP data from three F2 mapping populations and spanned a length of 1,443.84 cM, with an average marker spacing of 0.28 cM (Talukder et al. 2014). In the current study, bioinformatics analysis was used in conjunction with the published sunflower genome reference sequence to improve map construction accuracy, resulting in the mapping of 6,136 markers to 17 LGs. The map described here spans a total of 2,221.86 cM, with an average inter-marker distance of 0.36 cM. Compared to the high-density genetic map previously reported (Talukder et al. 2014), this genetic map contains more markers and spans longer genetic distances.

One challenge to efficient mapping, segregation distortion, is ubiquitous in many species and is observed when the genotypic frequency of a marker deviates from the expected Mendelian ratio. This phenomenon mainly results from gametophyte and/or zygotic selection or chromosomal rearrangements (Menz et al. 2002). Indeed, studies have demonstrated that segregation distortion of a marker can occur in a non-random and consistent distribution pattern, suggesting that the elimination of gametes or zygotes by a lethal factor located within a neighboring region of the marker causes this phenomenon (Mace et al. 2009). In the present study, an F2 mapping population that was employed to construct a linkage map of 6,136 assigned markers only produced 25 markers (0.41%) that exhibited significant segregation distortion. The low ratio of segregation distortion observed here may be related to the population type and genetic relationships within the mapping population used in this study. A previous study had indicated that marker distorted regions were present within LGs 4, 9, 11, and 12 in a sunflower map constructed across an F2 population (Talukder et al. 2014). In the current study, segregation distortion markers were also distributed on LG4, LG9, and LG11, which indicates that a similar mechanism of skewed segregation may be common to the two studies. Meanwhile, other studies have demonstrated that linkage mapping applications (such as QTL mapping) are not affected by the presence of segregation distortion markers (Xu and Hu 2009, Zhang et al. 2010) and thus may not negatively impact the successful application of high-density genetic map, as developed in this work.

The genetic map described in this study should be useful for three applications. First, the map provides useful data for further QTL mapping. Second, the results of this study should guide molecular marker-assisted breeding of sunflower germplasm and the cloning of related genes. Third, the high-quality genetic map described here will serve as a reference to anchor scaffolds onto chromosomes that will facilitate completion of next-generation sequencing of whole sunflower genomes (Badouin et al. 2017). However, abundant repetitive DNA sequences in sunflower may still hinder assembly accuracy.

Agronomic traits such as plant height, head diameter, and stem diameter are important traits for sunflower breeding. However, genetic studies focused on these traits in sunflower have seldom been reported. Using F2 and F3 populations, 3 significant QTLs for plant height were previously identified that were localized to LG1, LG5, and LG8 (Bert et al. 2003). In addition, 5 QTLs for head height, 4 QTLs for head diameter, and 5 QTLs for stem diameter have been identified using RILs (Al-Chaarani et al. 2004), while 6 QTLs for plant height were obtained using F2 and F3 populations and were localized to LG8 and LG17 (Yue et al. 2008), 3 QTLs for head diameter were obtained using F2 and F3 populations and were localized to LG9, LG13, and LG17 (Yue et al. 2009), and 11 QTLs for plant height and 9 QTLs for head diameter were obtained using RILs (Haddadi et al. 2011).

It has been acknowledged that sunflower oil with high oleic acid content shares positive nutritional qualities with olive oil (Lacombe et al. 2009). Therefore, one important focus of sunflower breeding would be to increase oleic acid content in seed oil. The genetics of oleic acid content in sunflower have been reported by many researchers (Al-Chaarani et al. 2004, Burke et al. 2005, Ebrahim et al. 2008, Fernandez-Martinez et al. 1989, Hongtrakul et al. 1998, Lacombe and Berville 2001, Miller et al. 1987, Perez-Vich et al. 2002). To date, these studies have resulted in the detection of several QTLs influencing oleic acid content (Kusterer et al. 2004, Premnath et al. 2016).

Here we identified 8 QTLs for plant height, head diameter, stem diameter, and oleic acid content. The QTL for stem diameter (SD_1) accounted for more than 15% of phenotypic variation in this study and will be useful in sunflower plant breeding. Unfortunately, it is difficult to compare QTLs found here with those described in previous reports, due to the unrelated markers used for map construction. However, some QTLs detected in this study, such as PH_1 on LG 7, PH_2 on LG 17, HD_1 on LG 5, HD_2 on LG 9, and OAC_1 on LG 9 share LGs with QTLs previously reported.
Meanwhile, three agronomic traits (PH, HD, and SD) in the F2 population exhibited normal distribution frequencies in this study, which confirmed that these traits are inherited in a quantitative manner (Fig. 4). However, oleic acid content did not exhibit a normal distribution frequency when we performed QTL mapping for this trait. This result may reflect the fact that a quantitative trait aligns with a normal distribution only under the hypothesis of multiple gene involvement. When the number of QTLs is small and there are a few QTLs with large genetic effects, phenotypic data do not obey a normal distribution, even though the non-normality of phenotypic data does not affect QTL mapping (Li et al. 2010).

In this study, LOD scores varied from 2.15 to 2.78 among QTLs identified for HP, HD, SD, and OAC and were thus low enough to prompt questioning of the accuracy of QTL identification. In our opinion, there are several reasons for the low LOD scores. First of all, low LOD scores were likely due to the small number of F2 individuals used in this study, resulting in reduced detection of small recombination rates and ultimately decreased QTL detection efficiency. In order to verify accuracy of these QTLs and detect the stability of QTLs in this study, next we will perform backcross tests in this population and QTL analysis for plants grown in different environments. Second, in our study we used R/qtl to analyze QTLs, which employs a reference coefficient that differs from that employed by other software (such as MapQTL), contributing to the low LOD scores observed for our QTL analysis results. Third, phenotypic data itself might contain a certain error due to an unknown but important factor that affects LOD scores. Nevertheless, the QTLs identified in this work will be helpful for future mapping of useful sunflower genes.

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