Construction of a High Density Genetic Map and QTL Mapping of PEG-Induced Drought Tolerance at Seedling Stage in Sesame Using Whole Genome Re-Sequencing

Junchao Liang

Crop Research Insitute, Jiangxi Academy of Agricultural Sciences, Nanchang, China   https://orcid.org/0000-0003-0635-5243

Yanying Ye

Crop Research Institute, Jiangxi Academy of Agricultural Sciences, Nanchang, China

Xiaowen Yan

Crop Research Institute, Jiangxi Academy of Agricultural Sciences, Nanchang, China

Tingxian Yan

Crop Research Institute, Jiangxi Academy of Agricultural Sciences, Nanchang, China

Yueliang Rao

Crop Research Institute, Jiangxi Academy of Agricultural Sciences, Nanchang, China

Hongying Zhou

Nanchang Institute of Technology

Jian Sun (✉ whsunjian@aliyun.com)

Nanchang Branch of National Center of Oilcrops Improvement   https://orcid.org/0000-0002-7443-179X

Meiwang Le

Crop Research Institue, Jiangxi Academy of Agricultural Sciences, Nanchang, China

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Abstract

Background

Improvement in sesame (*Sesamum indicum* L.) drought tolerance at seedling stage is important for yield stability. Genetic approaches combing with conventional breeding is the most effective way to develop drought-tolerant cultivars. So far, very few studies have been reported to reveal gene/ quantitative trait loci (QTL) controlling drought tolerance in sesame. To identify the genomic regions associated with drought tolerance, we constructed a high-density genetic map using a recombinant inbred line (RIL) population through whole genome re-sequencing (WGRS) technique. QTLs contributing to three seedling traits were identified under both non-stress and water stress conditions.

Results

Three drought tolerance related traits and their relative values (the ratio of value under stress to value under control condition), including seedling weight (SW), shoot length (SL) and root length (RL), were evaluated under control and PEG-induced osmotic conditions at seedling stage in a RIL population derived from cross of Zhushanbai (ZSB) and Jinhuangma (JHM). Significant variation and high broad sense heritability were observed for all traits except SW under stress condition in the population. With this population, a high-density linkage map with 1354 bin markers was constructed through WGRS strategy. Composite interval mapping analysis was performed for all the traits as well as their relative phenotypic data. A total of 34 QTLs were detected for these three traits under both conditions and their relative values, and 13 stable QTLs associated with seven traits could be revealed in two independent experiments, explaining on average, 4.95-16.26% of phenotypic variation for each QTL. Four of them contributed more than 10% of phenotypic variation. Root length related QTLs were first identified in sesame. One region on chromosome 12 contained two major QTLs related to RL under osmotic condition and relative RL.

Conclusion

The current study reports the first QTL mapping of drought tolerance related traits through a RIL population and first QTL detection of root related trait (root length) in sesame. These findings will provide new genetic resources for molecular improvement of drought tolerance and candidate gene identification in sesame.

Background

Drought stress usually refers to a water shortage which causes dramatic morphological, biochemical, physiological, and molecular changes [1]. These changes would severely affect plant growth and crop yield stability. In past four decades, it is estimated that the drought has caused a cereal loss of 1820 million Mg [2]. Meanwhile, the continuous global warming and climate change will probably increase the frequency of drought. Sesame (*Sesamum indicum* L., 2n = 26) is one of the most important oilseed crops in the world. Sesame seeds are known to be rich in protein, vitamins and special antioxidants, such as sesamin and sesamolin, which make sesame become a very healthy food favored by consumers. Comparison with most of other oilseed crops, sesame is considered as a resilient crop that is more tolerant to drought stress. However, sesame growth and productivity are vulnerable to severe drought stress, especially in the arid and semi-arid areas. Sesame belongs to shallow root plants and is very sensitive to drought stress during the germination and flowering stages [3]. Improvement of drought tolerance at these two stages of sesame is very important for yield stability.

Drought tolerance is a complex quantitative trait in plants. QTL mapping and genome-wide association study (GWAS) have been widely used for genetic analysis of drought tolerance related traits in many plants, such as rice [4, 5], wheat [6–8], cotton [9, 10] and *Brassica napus* [11]. These traits including root length, coleoptile length and shoot length for

seedling stage, and yield-related traits for flowering stage. A solution of polyethylene glycol (PEG) is frequently used to simulate drought stress through treating the seeds with the PEG solution for days, especially in germination or seedling stress experiments [5, 6, 12].

In sesame, several studies have reported that many traits including germination rate, seedling growth, shoot length, root length and yield related traits could be affect by drought stress [13–15]. Mensah et al. [13] used varying PEG concentrations to simulate drought effect on germination of sesame and found that higher osmotic conditions (0.25–0.50 MPa) significantly reduced the germination rate, radical and shoot development, but lower osmotic tensions (0.0625 MPa) could enhance root growth. Only few studies have been conducted yet on genetic analysis of drought tolerance. Li et al. [16] confirmed 15% PEG 6000 as a suitable concentration for examining drought tolerance in sesame germplasms, and performed a GWAS of stress tolerance indexes related to NaCl-salt and PEG-drought at the germination stage with 490 sesame accessions, and identified nine and 15 QTLs for drought and salt stresses, respectively. Ten stable QTLs were also identified for five drought related traits at the sesame flowering stage through GWAS [17]. By using gene association study, gene expression and transgenic experiments, a candidate gene *SiSAM* was identified to confer drought tolerance by modulating polyamine levels and ROS homeostasis in sesame [17]. So far, there is no study on QTL mapping for drought tolerance traits using bi-parental population in sesame.

The recent rapid development of high throughput sequencing makes it easier to construct high-density genetic map with numerous single-nucleotide polymorphism (SNP) markers. SNPs are the most abundant form of genetic variation throughout the genomes and are ideal genetic markers for genetic and breeding applications. Recent released sesame reference genome has greatly helped in SNPs identification in sesame through multiple next generation sequencing strategies, including genotyping by sequencing (GBS), reduced representation sequencing (RAD) and whole genome re-sequencing. These methods have been successfully utilized for high resolution mapping of QTLs in sesame [18–22].

To further explore the genetic foundation of sesame drought tolerance, in the present study, we developed a high-density genetic map through WGRS of 180 RILs generated by two sesame landraces Jinhuangma (JHM) and Zhushanbai (ZSB) from China. By performing PEG stress experiments, QTLs for fresh seedling weight, shoot length and root length were identified under control and stress conditions. These can provide a valuable contribution to understand the genetic basis of drought tolerance and facilitate marker-assisted breeding for stress tolerance in sesame.

Results

Whole genome re-sequencing and genotyping

Using whole genome re-sequencing approach, we generated over 1.1 billion reads from two parents and 180 RILs, with an average of 6.3 million reads per RIL, providing an average read depth of 4.18×. For the two parents JHM and ZSB, ~14.6 million and ~13.9 million reads were obtained respectively. All the clean reads were mapped to the sesame reference genome Zhongzhi No. 13 [19] using BWA. After filtering, a total of 466,911 high-quality SNPs and 72,981 InDels (Insertion and deletions) were identified among the RILs. Chromosome (chr) 3 harbored the largest number of SNPs and InDels, while chromosome 7 contained the fewest number of variants. The density of the SNP and InDel loci in the genome was 1788.15/Mb and 280.32/Mb respectively (Additional file 1: Table S1).

High density genetic map construction

All the filtered SNPs/InDels were used to construct bin-map through MPR method. A total of 1354 bin markers covering 538,090 variants were identified on the 13 chromosomes (Fig. 1). By using genotype data of the RIL population, we

construct a high-density genetic map with a total genetic distance of 1295.45 cM. The mapped bin per chromosome ranged from 58 (chr7) to 155 (chr3) with an average of 104.2 per chromosome (Fig. 2, Table 1). The density of bin markers in the whole genome was 0.98 cM/locus, covering an average physical length of 158.74 kb per bin.

Table 1 Characteristics of the high-density genetic map

Chr.[a]	Length(cM)	No. markers	No. bins	Average bin interval (cM)	Max interval (cM)
chr1	89.539	61646	125	0.722	4.528
chr2	161.486	38457	120	1.357	11.684
chr3	128.823	76256	155	0.837	5.969
chr4	108.981	26753	77	1.434	25.697
chr5	116.469	43314	97	1.213	15.049
chr6	114.018	60058	136	0.845	6.339
chr7	55.011	12058	58	0.965	3.833
chr8	111.070	45042	119	0.941	10.355
chr9	91.418	37416	114	0.809	9.503
chr10	123.251	36959	105	1.185	29.912
chr11	65.639	40345	85	0.781	6.339
chr12	71.325	31949	77	0.938	9.503
chr13	58.423	27837	86	0.687	3.491
Whole	1295.453	538090	1354	0.978	10.939

[a]Chr., Chromosome

In addition to several gaps of more than 10 cM identified on chr2 (1), chr4 (1), chr5 (3), chr8 (1) and chr10 (2), the bin markers were distributed evenly along 13 chromosomes. The chromosome with the longest genetic length was chr2, which contained 120 bin markers covering a genetic length of 161.49 cM. Chr7 covered the shortest genetic length (55.01 cM) with 58 bin markers. A total of 1286 (95.0%) bin markers were less than 500 kb in length, and 40 bins covered physical length larger than 1 Mb. The largest bin located on chromosome 9 (c09b114), with a physical length larger than 5 Mb. (Addidtional file 2: Table S2).

To evaluate the quality of this high density genetic map, we investigated the colliearity between this genetic map and physical map. The dot plot of markers in the 13 linkage groups aligned well with the Zhongzhi No.13 reference chromosome, indicating excellent colliearity between genetic map and physical map (Fig. 3).

Phenotypic variation and correlation analysis

The phenotype data analysis showed that each trait varied among two parents and different RILs in both treatments (Fig. 4; Table 2). The values of traits root length (RL), shoot length (SL) and fresh seedling weight (SW) of parent JHM were all lower than ZSB to some extent. The phenotypic distributions of mean showed continuous variations and transgressive segregations on both directions of the parents, suggesting polygenic inheritance of all traits in sesame. In the PEG osmotic condition, mean SL and SW of the RIL population were significant inhibited and reduced by 29.3% and

37.1% respectively when compared with the control condition, whereas the mean RL trait was slightly affected and reduced by 2.7%.

To better understand the responses of these traits to drought stress, we also investigated the relative phenotypic data of these three traits (Fig. 5; Table 2). The mean values of relative SW (RSW) and SL (RSL) showed no differences between two parents and mean relative RL (RRL) of ZSB (0.88) was significantly higher than that of JHM (0.58). In RILs, highly significant differences were also noted for all three relative parameters. Among them, RRL showed remarkable variation, ranging part from 1 in two directions, indicating the inhibition or induction of osmotic stress on root growth in the population. The broad sense heritability of all traits under both conditions ranged from 34% to 88%, with the highest heritability values recorded for root length under PEG osmotic condition (88%), and relative seedling weight had the lowest value of 34% heritability (Table 2).

Table 2 Phenotypic data and heritability of six traits in parents and RIL population

Traits	Experiment		ZSB	JHM	RIL mean	RIL range	h^2
Seedling weight (mg)	1	Control	73.35	62.10	60.41	46.56-76.50	0.62
	2	Control	73.13	62.75	66.36	39.40-79.88	
	1	PEG	40.65	35.20	38.87	29.67-58.08	0.55
	2	PEG	41.95	35.25	40.89	12.50-60.93	
Shoot length (cm)	1	Control	5.04	4.88	4.61	3.77-5.47	0.73
	2	Control	5.22	4.94	4.84	3.93-6.56	
	1	PEG	3.18	3.16	3.27	2.67-3.86	0.71
	2	PEG	3.19	3.09	3.41	2.55-3.98	
Root length (cm)	1	Control	7.48	5.78	6.63	4.94-8.18	0.71
	2	Control	7.46	5.86	6.15	3.91-7.43	
	1	PEG	6.48	3.30	6.34	3.24-8.67	0.88
	2	PEG	6.59	3.49	6.09	3.19-8.75	
Relative seedling weight	1		0.55	0.57	0.64	0.53-0.95	0.34
	2		0.57	0.56	0.65	0.16-1.02	
Relative shoot length	1		0.63	0.65	0.71	0.56-0.87	0.70
	2		0.61	0.62	0.71	0.53-0.91	
Relative root length	1		0.87	0.57	0.96	0.61-1.33	0.75
	2		0.88	0.59	0.97	0.58-1.41	

Correlations among seedling weight, shoot length and root length in both conditions were also surveyed (Table 3). For control condition, all the three traits (herein abbr. SWC, SLC and RLC, repectively) were positively correlated with each other in the two trials. Correlations among SW, SL and RL under PEG stress condition (herein abbr. SWP, SLP and RLP, respectively) were weaker than that under control condition. RLP were positively correlated with SLP in one or two experiments, but SWP had no significant relationships with RLP (significant at $P = 0.05$) in either of two trials. All the

identical traits showed significant correlation between control and stress condition, with the average correlation coefficients ranging from 0.347 for SL to 0.472 for RL.

Table 3 Correlation coefficents for six traits in the RIL population

	SW	SL	RL	SWP	SLP	RLP
SW	1					
SL	0.193** 0.390**	1				
RL	0.295** 0.381**	0.249** 0.475**	1			
SWP	0.776** 0.461**	-0.008 0.042	0.240** 0.086	1		
SLP	0.031 0.288**	0.303** 0.390**	0.102 0.218**	0.091 0.356**	1	
RLP	0.001 0.117	0.172* 0.380**	0.454** 0.489**	-0.099 0.072	0.414** 0.509**	1

The correlation coefficients from experiment 1 and experiment 2 are shown in the first and second rows, respectively

* and ** indicates significance at P = 0.05 and 0.01, respectively

QTL mapping

Composite interval mapping was used to identified chromosome regions associated with seedling weight, shoot length and root length under control and PEG stress conditions. In total, 34 QTLs were detected for all the traits under both conditions including the relative traits values, and 13 of them could be detected under two experiments. These stable QTLs were mapped to six chromosomes.

QTL identification under control condition

Under control condition, eleven QTLs were identified as being associated with these traits (Table 4). *qSLC1*, which could be identified in both experiments, influenced shoot length, located at chromosome 1, had the highest LOD value, explaining an average of 16.26% of the phenotypic variation. Three chromosome regions showed associated with SW. Among them, *qSWC12* was mapped to chromosome 12 and expressed stably across the trials, contributing to higher SW through JHM alleles. For shoot length, besides *qSLC1*, another two stable QTLs were detected on chromosome 5 and 12. One of them,

Table 4 QTLs identified for drought tolerance traits

Traits	Chromosome location	Locus	Flanking Markers	Experiment 1 (2018)			Experiment 2 (2019)		
				LOD[a]	AE[b]	R^2(%)	LOD[a]	AE[b]	R^2(%)
SW-control	Chr2	*qSWC2*	c02b067-c02b073				3.22	1.71	6.54
	Chr8	*qSWC8*	c08b086-c08b090	3.75	1.62	7.17			
	Chr12	*qSWC12*	c12b069-c12b071	5.56	1.99	10.82	2.98	1.56	6.05
SL-control	Chr1	*qSLC1*	c01b092-c01b100	9.06	-0.14	16.38	8.72	-0.17	16.14
	Chr5	*qSLC5*	c05b087-c05b094	3.05	0.08	5.02	2.87	0.10	4.87
	Chr8	*qSLC8*	c08b065-c08b070	3.54	0.08	5.86			
	Chr12	*qSLC12*	c12b062-c12b072	5.34	0.10	9.06	5.30	0.13	9.38
RL-control	Chr1	*qRLC1*	c01b086-c01b087				5.23	-0.20	10.46
	Chr4	*qRLC4*	c04b040-c04b046	4.91	-0.21	9.12	4.54	-0.19	8.33
	Chr6	*qRLC6*	c06b129-c06b135				4.57	0.18	8.46
	Chr10	*qRLC10*	c10b076-c10b080	4.62	-0.20	8.40			
SW-PEG	Chr1	*qSWP1*	c01b003-c01b010	4.20	-1.23	8.20			
	Chr3	*qSWP3*	c03b116-c03b119				3.16	1.39	6.61
	Chr9	*qSWP9*	c09b031-c09b040				3.57	-1.62	7.50
SL-PEG	Chr1	*qSLP1*	c01b032-c01b035	4.27	-0.07	8.26			
	Chr8	*qSLP8*	c08b055-c08b063	3.06	0.06	5.88	3.04	0.07	5.83
	Chr9	*qSLP9-1*	c09b015-c09b021				3.76	0.10	7.26
	Chr9	*qSLP9-2*	c09b031-c09b033				5.56	-0.12	11.00
RL-PEG	Chr1	*qRLP1*	c01b062-c01b070	4.97	-0.31	7.97	4.36	-0.31	6.77
	Chr6	*qRLP6*	c06b054-c06b060	3.28	0.26	5.15			

	Chr	QTL	Interval	LOD	Add	PVE	LOD	Add	PVE
	Chr7	*qRLP7*	c07b030-c07b036	4.37	-0.29	6.61	4.14	-0.30	6.40
	Chr12	*qRLP12*	c12b032-c12b036	7.17	-0.38	11.85	8.78	-0.45	14.46
RSW	Chr5	*qRSW5-1*	c05b019-c05b029	3.74	0.01	6.80			
	Chr5	*qRSW5-2*	c05b071-c05b074				3.01	0.02	6.33
	Chr6	*qRSW6*	c06b041-c06b045	8.29	0.01	8.29			
	Chr12	*qRSW12*	c12b061-c12b072				3.56	-0.02	7.47
RSL	Chr1	*qRSL1-1*	c01b035-c01b049				3.56	-0.17	6.39
	Chr1	*qRSL1-2*	c01b109-c01b113	5.37	0.02	9.48	6.67	0.02	12.15
	Chr11	*qRSL11*	c11b044-c11b051	3.74	0.02	6.36			
RRL	Chr1	*qRRL1*	c01b052-c01b063	4.12	-0.04	6.56	3.92	-0.04	6.42
	Chr3	*qRRL3-1*	c03b043-c03b055				3.65	-0.04	5.97
	Chr3	*qRRL3-2*	c03b102-c03b113	4.29	-0.04	6.82			
	Chr7	*qRRL7*	c07b020-c07b028	4.45	-0.04	7.09	3.19	-0.04	5.14
	Chr12	*qRRL12*	c12b032-c12b036	6.26	-0.05	10.26	9.36	-0.07	16.47

[a]LOD: likelihood of the odds

[b]Additive effect: positive and negtive indicated ZSB and JHM allele produced larger value respectively.

[c]E followed by 1 or 2 designate two independent experiments

qSLC12, was mapped to the interval (c12b062-c12b072) on chromosome overlapped with the interval of *qSWC12* (c12b069-c12b071) on chromosome 12. *qSLC12* had the second highest LOD value and explained up to 9.38% of phenotypic variation in SL. *qSLC1* gained favourable allele from ZSB, while *qSLC5* and *qSLC12* were associated with increased SL through JHM alleles.

A total of four root length QTLs were detected under control condition. One stable QTL (*qRLC4*) located on chromosome 4 were detected in two trials (Table 4), had an average LOD value of 4.73 and explained at least 8.33% of phenotypic variation. The ZSB allele of *qRLC4* was associated with longer root length.

QTL identification under PEG stress condition

Eleven chromosome regions were found to be associated with SW, SL and RL under PEG stress condition (SWP, SLP and RLP) (Table 4). For SWP, two QTLs were identified and mapped on chromosome 3 and 9 (*qSWP3* and *qSWP9*), but none of them could be detected across two trials. Four chromosome regions were detected to be associated with SL. Two of them located on chromosome 9. One QTL located on chromosome 8 (*qSLP8*) were steadily expressed in both trials and explained an average of 5.86% of phenotypic variation. *qSLP8* increased SL under osmotic condition through the ZSB allele.

Of the four QTLs associated with RL under PEG stress condition, three could be identified in two experiments (Table 4). Among them, *qRLP12* had the highest LOD value and strongest effect on RL, explaining up to 14.46% of phenotypic variation. The other two QTLs were located on chromosome 1 (*qRLP1*) and 7 (*qRLP7*), explaining at least 4.36% and 4.14% of phenotypic variation respectively. The ZSB alleles of these three stable QTLs were associated with longer root under PEG stress condition.

QTL identification for drought tolerance index

Drought tolerance index was defined as relative traits value in this study. Twelve chromosome regions were identified to be associated with relative seedling weight (RSW), relative shoot length (RSL) and relative root length (RRL) (Table 4). For RSL, *qRSL1-2* detected in both trials had the strongest effect on RSL, explaining an average of 10.82% phenotypic variation, with LOD scores > 5. JHM contributed the positive alleles for *qRSW5* and *qRSL1-2*. Four QTLs were identified for RSW in one of the trials. No stable QTL was detected for RSW.

Among the five chromosome regions associated with RRL, *qRRL12* had the strongest effect and explained, on average, 13.37% of the phenotypic variation in two trials, with an average LOD score of 7.81. This QTL was mapped to the same chromosome region as *qRLP12* (Fig. 6; Table 4). Besides *qRRL12*, two QTLs, *qRRL1* and *qRRL7*, contributed at least 6.42% and 5.14% of the phenotypic variation in two trials, respectively. All of these three stable QTLs were responsible for the increase of RRL through the ZSB allele.

Discussion

Sesame is one of the most important oil crops worldwide and provides sorts of specific lignins which are very good for human health. Drought is a major stress effecting sesame growth at seedling stage. Efforts have been made for drought tolerance QTL and candidate genes identification in recent years [5, 16]. However, the drought tolerance related traits are quite complex and controlled by multiple genes and environmental factors. Very few genetic studies were performed for drought tolerance at sesame seedling stage, especially for root related traits and no QTL was reported. In this study, 180 RILs were used to identify genomic regions associated with PEG-induced drought tolerance traits at early seedling stage in sesame. Seedling weight, shoot length and root length were evaluated under both control and stress conditions. Through whole genome re-sequencing, a high-density genetic map was constructed. 13 stable QTLs associated with drought tolerance traits were detected.

In recent decades, with the development of next-generation sequencing (NGS) technology and the release of whole genome sequence of sesame, numerous SNP markers have been used for QTL genetic mapping and GWAS analysis. Bin-maps constructed by high-density SNP markers using NGS have been widely used in sesame QTL mapping [21, 23]. In the present study, we re-sequenced each RIL with an average read depth of 4.18 × and construct a high-density genetic map with 1354 bins on all 13 chromosomes. The average genetic length was 0.978 cM per bin, which was similar with previous maps [21, 23]. Comparing with other sesame genetic maps developed by restriction-site associated DNA sequencing (RAD-seq) or GBS approach, WGS strategy could acquire much more variants. We obtained 466,911 high-quality SNPs and 72,981 InDels for bin markers assignment, which was more than 30 times of

13,679 SNPs identified by GBS in Zhang et al. [23] and 11,924 SNPs detected by RAD-seq in Zhang et al. [21]. The high-density variants could improve the resolution of QTL and also help perform fine mapping of QTL controlling various agronomic traits.

For drought tolerance evaluation, due to the unpredictability of rainfall and soil heterogeneity of field experiment, PEG solution has been widely employed as an alternative to simulate water shortage condition, especially for the trials at seedling stage [6, 16, 24]. In sesame, Li et al. [16] used relative values of germination rate and fresh weight (the ratio of the traits value under stress conditions to the same traits under stress free conditions) to evaluate the drought tolerance of sesame at early seedling stage. For drought tolerance at sesame flowering stage, Dossa et al. [17] investigated wilting level of the whole plant, stem length, and some yield related traits. However, no comprehensive genetic study has been conducted yet for the root traits under desiccation stress condition in sesame. In this study, three characters including SW, SL and RL were measured in the RIL population under control and PEG stress condition. All the measured traits showed significant genetic variation under both conditions, which enable the identification of QTLs associated with traits in sesame. We found high level of broad heritability of the trait RL under stress condition, indicating the selection of this trait could be effective in the breeding program. Relative performances of trait values were usually used as indicators for the evaluation of stress tolerance. We also investigated SW, SL and RL in current study and the results showed that relative RL distributed part from 1 in both directions, suggesting the PEG solution treatment in our study only inhibited the root growth of some lines. This also indicated that the different performances of root length in the RILs depended on genotypic variation.

In this study, QTLs were identified for all the traits under control and osmotic stress conditions. For root length, we found that in ZSB, both *qRLP12* and *qRRL12* were mapped to the same interval on chromosome 12, associated with increased RL under PEG stress condition and RRL. *qRLP12* and *qRRL12* had the highest LOD values of all the QTLs associated with RLP and RRL respectively, and explained up to 14.46 and 16.67% of the phenotypic variation. These results implied that this interval on chromosome 12 contains a major QTL that associated with root length and drought tolerance. To our knowledge, this is the first report of QTLs that associated with root length in sesame. Since the root is the tissue responsible for water uptake, deep root system can help plants absorbing water from deep soil layers to avoid drought stress. This interval in ZSB could be useful for drought tolerance breeding in sesame. In addition to *qRLP12*, the interval of other two stable QTLs controlling root length under osmotic stress condition were both closely linked with that of QTLs associated with relative root length (*qRLP1* and *qRRL1*, *qRLP7* and *qRRL7*) (Fig. 6). This strongly indicated close relationship between traits RLP and RRL, which may enable selection for the complex drought tolerance trait through an easily observable related trait, such as root length.

The traits of above ground part also play important role for avoiding drought. Long shoot is conducive to rapid seedling formation and allows deeper sowing. In this study, eight QTLs were identified for SL under both conditions, four of them (*qSLC1*, *qSLC5*, *qSLC12* and *qSLP8*) stably expressed in two trials. For SW, only one major QTL *qSWC12* for SW under control condition was detected in two trials. We could not detect any stable QTL for trait SW under osmotic stress condition and trait RSW, which may be due to their relative low heritabilities. This indicated that seedling weight may not be suitable as selection trait for drought tolerance in seedling stage of sesame. *qSWC12* and *qSLC12* was both located on similar region of chromosome 12. QTLs for shoot length and seedling weight co-location on chromosomes have also been report in soybean [25]. The JHM alleles for *qSWC12* and *qSLC12* were related to increased SW and SL under control condition, indicating the parent with lower phenotypic values may also contain favorable alleles for traits SW and SL. QTL cluster in this region could be raised by both linkage and pleiotropic effects.

Based on the QTL mapping results, we examined the genes located within the genomic region of *qRLP12/qRRL12*, which had the largest effect on drought tolerance in this study. This region was related with both RL under PEG stress condition and relative RL. 55 annotated genes were found between the boundary markers of the interval of

qRLP12/ qRRL12 in the sesame reference genome (Additional file 3: Table S3). Then, we identified homologs of these genes in *Arabidopsis thaliana* through BLASTX using the coding sequences of these 55 genes. Of them, 43 could find homologs in *A. thaliana*, and three genes may be identified as candidates. *SIN_1022439* encodes an uncharacterized peptide, which is homologous with *AT1G17120* (RASD1, Responsiveness to ABA salt and drought 1). It had been reported that RASD1 was expressed predominantly in the vascular system, and *rasd1* mutant lines were verified to be drought intolerant and less sensitive to exogenous ABA and NaCl during germination and root growth than wild-type plants [26]. *SIN_1022428*, homologous with *AT1G06490*, encoding callose synthase 7 (CalS7), which evolves in callose deposition in developing sieve elements during phloem formation. The 5-day-old seedling of *AT1G06490* mutant developed much shorter roots than wild-type [27]. *SIN_1022433* is another candidate encoding subtilisin-like protease. Its homolog in *A. thaliana* (*AT2G04160*, AIR3) was reported to be involved in lateral root morphogenesis and induced by NAC genes which regulated multiple abiotic stress responses [28, 29]. The confirmation of candidate genes needs further fine mapping of this QTL.

Conclusions

In conclusion, we performed first QTL mapping of drought tolerance related traits using a RIL population and identified root length QTLs for the first time in sesame. By using PEG treatment, inheritances of three traits including seedling weight, shoot length and root length were interpreted. Root length had the largest broad sense heritability, while seedling weight occupied the lowest position. Using WGRS technique, we identified a total of 13 stable QTLs for drought tolerance related trait at seedling stage of sesame. Four of them explained more than 10% phenotypic variation and had an LOD score larger than 6. The current study was the first report for QTL identification for root length, which is considered as one of the most important traits for drought tolerance in plant. A genomic interval on chromosome 12 contained two QTLs associated to RLP and RRL. We identified three genes that may be related to the regulation of root length under osmotic stress. The major QTL regions and linked markers can provide potential genetic resources for molecular marker-assisted selection and further cloning of functional genes for drought tolerance in sesame.

Methods

Plant materials and phenotyping

The *Sesamum indicum* L. cultivars Jinhuangma (JHM) and Zhushanbai (ZSB) were landraces collected at Jiangxi province and Hubei province respectively of China. The seeds of JHM and ZSB used in this study were obtained from sesame germplasm reservoir of Nanchang Branch of National Center of Oilcrops Improvement, Jiangxi Academy of Agricultural Sciences, China. A set of 180 F_9 recombinant inbred lines developed by single seed descent from the cross of JHM and ZSB was used for QTL mapping. All the plants were grown in a nylon net house to prevent cross-pollination caused by insects.

Polyethylene glycol (PEG)-simulated drought stress trials were performed by using both parent and RIL population in two years. For each line, 50 mature seeds were germinated on two layers of filter paper in each plastic container (10 cm × 10 cm × 5 cm) with top. The plastic containers were maintained under dark conditions and a constant temperature of 28 °C in climatic chamber for five days. For RIL population, two treatments were performed at the same time. In control condition, 40 mL ddH$_2$O was added into each box while in PEG stress condition 40 mL of 15% w/v PEG6000/water solution was applied. Three independent biological replicates were performed for each genotype in both conditions. Two independent experiments were conducted in two years (2018 and 2019). 20 normal plants per genotype were phenotyped in each condition of each replication five days after germination. Data of root length (RL), shoot length (SL) and fresh seedling weight (SW) were recorded on individual plants. Drought tolerance index was defined as

relative traits value, which was estimated as the ratio of the traits value under PEG stress to the traits value under control condition. For each trial, all the phenotypic data were analyzed using the mean value of three replicates. Statistical analysis of phenotype data and Pearson correlation analysis were carried out using SPSS statistical package (SPSS Inc., Chicago, IL).

Dna Extraction, Sequencing And Snp/indel Discovery

Total genomic DNA of JHM and ZSB along with 180 RILs were extracted from fresh young leaves following the standard protocols with the plant genomic DNA purification kit (B518261, Sangon Biotech Co., Ltd., Shanghai). Paired-end sequencing libraries with 300–500 bp insertion were constructed for each DNA samples. The libraries were sequenced with 150 bp (PE150) read length by using an Illumina Hiseq 2000 system (Illumina Inc., San Diego, CA, USA). The raw reads were filtered and aligned to the reference genome of sesame cultivar Zhongzhi No. 13 (Sinbase 2.0) [19] using the Burrows-Wheeler alignment (BWA) tool with default parameters [30]. SAMtools v1.9 [31] was used to convert sequence alignment map (SAM) format to binary alignment map (BAM) files. Aligned BAM files were sorted with SortSam in Picard (http://broadinstitute.github.io/picard/). SNP/InDel detection was performed by using HaplotypeCaller in Genome Analysis Toolkit (GATK) v4.0.11.0 [32].

Linkage Map Construction

The SNPs or InDels were filtered according to three criteria: (1) missing data rate < 20%; (2) minor allele frequency (MAF) > 0.2; and (3) loci that were homozygous in both parents and heterozygous in less than 15% of the RILs. Filtered SNPs/InDels were used to construct bin-map through maximum parsimonious inference of recombination (MPR) method described by Xie et al. [33]. First, all variants were re-filtered by permutations involving resampling of SNPs/InDels windows and then inferred by Bayesian method. The genotype of each locus in RILs was determined assisted by hidden Markov model. Consecutive SNPs/InDels sites with the same genotype as one parent were assembled into a block. A recombination event was defined as a transition between two blocks with different genotypes. The R/qtl package (http://rqtl.org/) was used to construct the linkage map.

Qtl Analysis

The broad sense heritability was estimated with the formula: , where σ^2_g is the estimated genetic variance and σ^2_e is experimental error. QTLs for each trait in each experiment were identified by composite interval mapping (CIM) method using Window QTL Cartographer v2.5 [34] (http://statgen.ncsu.edu/qtlcart/WQTLCart.htm). The standard model 6 and a window size of 10 cM was applied. The level of significance was determined with 1000 permutations, with a confidence level of 95%. The LOD score for declaring a QTL was 2.5 or above. MapChart software was used to construct the graphical representation of QTL positions [35].

Abbreviations

QTL
Quantitative trait loci; RIL:Recombinant inbred line; WGRS:Whole genome re-sequencing; SW:seedling weight; SL:shoot length; RL:root length; GWAS:genome-wide association study; PEG:polyethylene glycol; SNP:single-nucleotide polymorphism; GBS:genotyping by sequencing; RAD:reduced representation sequencing; JHM:Jinghuangma; ZSB:Zhushanbai; InDels:Insertion and deletions; chr:chromosome; RSW:Relative seedling weight; RSL:Relative shoot

length; RRL:Relative root length; SWC:SW under control condition; SWP:SW under PEG stress condition; SLC:SL under control condition; SLC:SL under control condition; SLP:SL under PEG stress condition; RLP:RL under PEG stress condition; LOD:likelihood of the odds; NGS:next-generation sequencing; RAD-seq:restriction-site associated DNA sequencing; MPR:maximum parsimonious inference of recombination; CIM:composite interval mapping

Declarations

Author information

Affiliations

Crop Research Institute, Nanchang Branch of National Center of Oilcrops Improvement, Jiangxi Province Key Laboratory of Oilcrops Biology, Jiangxi Academy of Agricultural Sciences, Nanchang, China

Junchao Liang, Jian Sun, Yanying Ye, Xiaowen Yan, Tingxian Yan, Yueliang Rao, Hongying Zhou & Meiwang Le

Authors Contributions

JL and JS designed and performed the experiment, and wrote the manuscript; JL and YY performed the experiment and drew the graphs; YY, TY, WY, YR and HZ constructed the population and collected and analyzed the data. MY designed and revised the manuscript; all the authors reviewed and approved this submission.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

We declare that these experiments comply with the ethical standards.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Conflict of Interest Statement:

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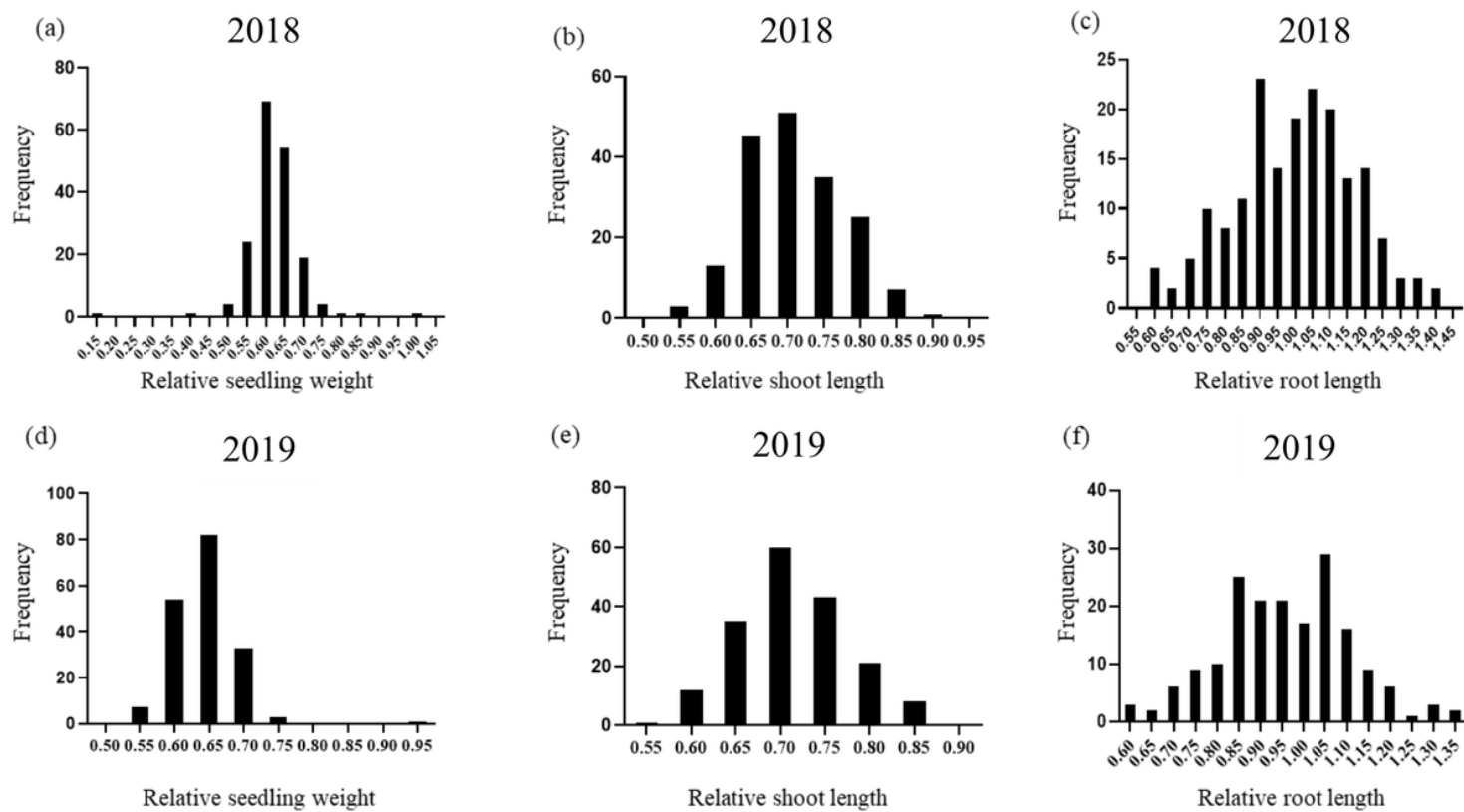
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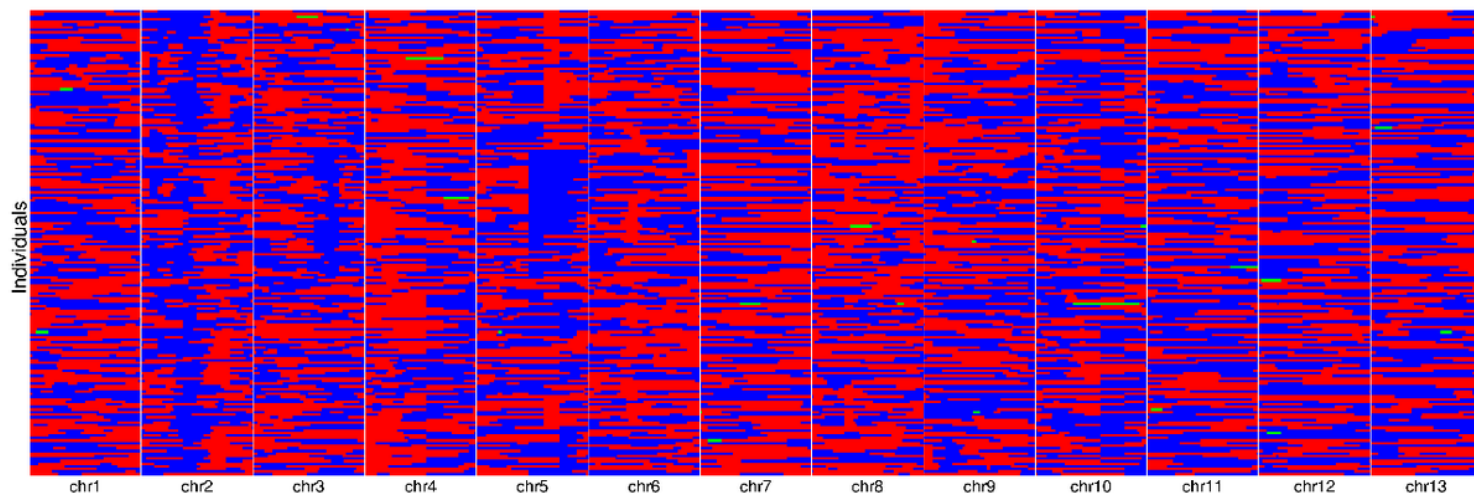
Figure 1

Genotype data of individuals of the RIL population, red and blue represent Zhushanbai and Jinhuangma, green represents heterzygote.
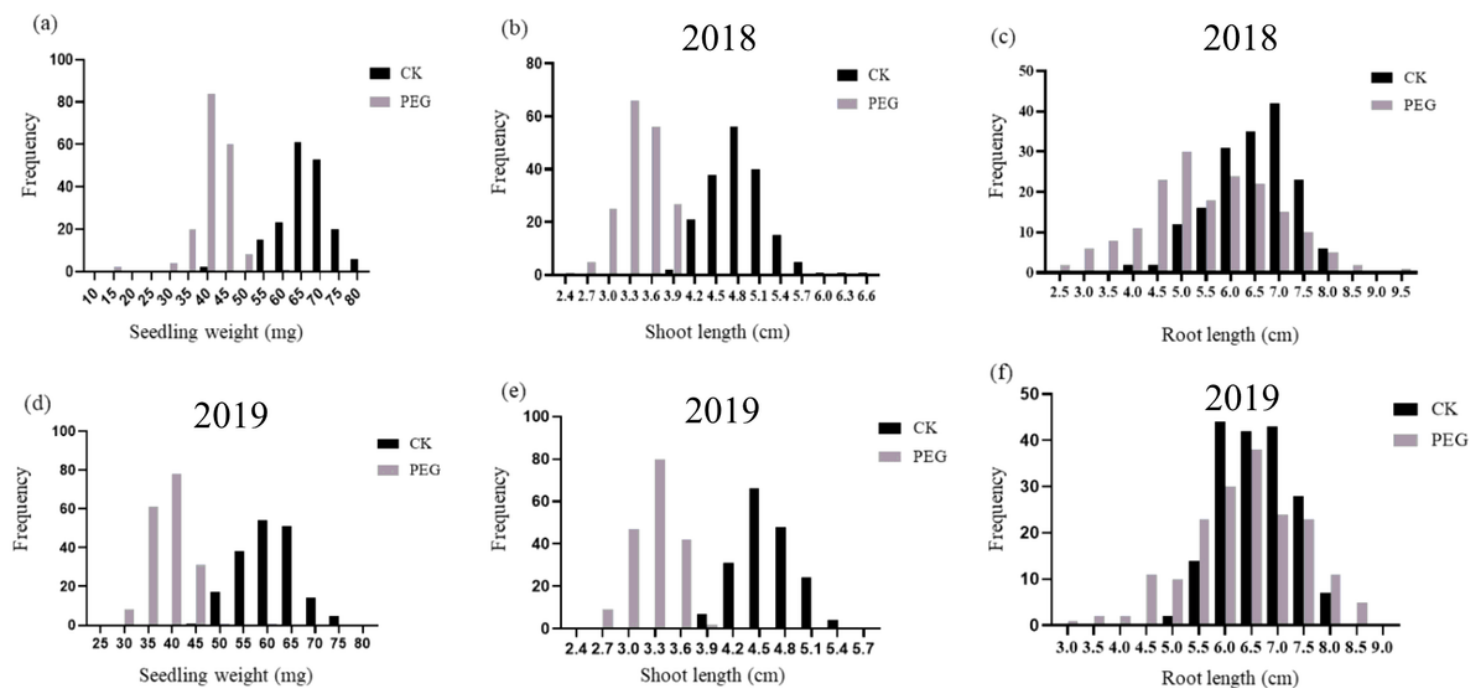
Figure 2

Distributions of bin markers on 13 chromosomes.

Figure 3

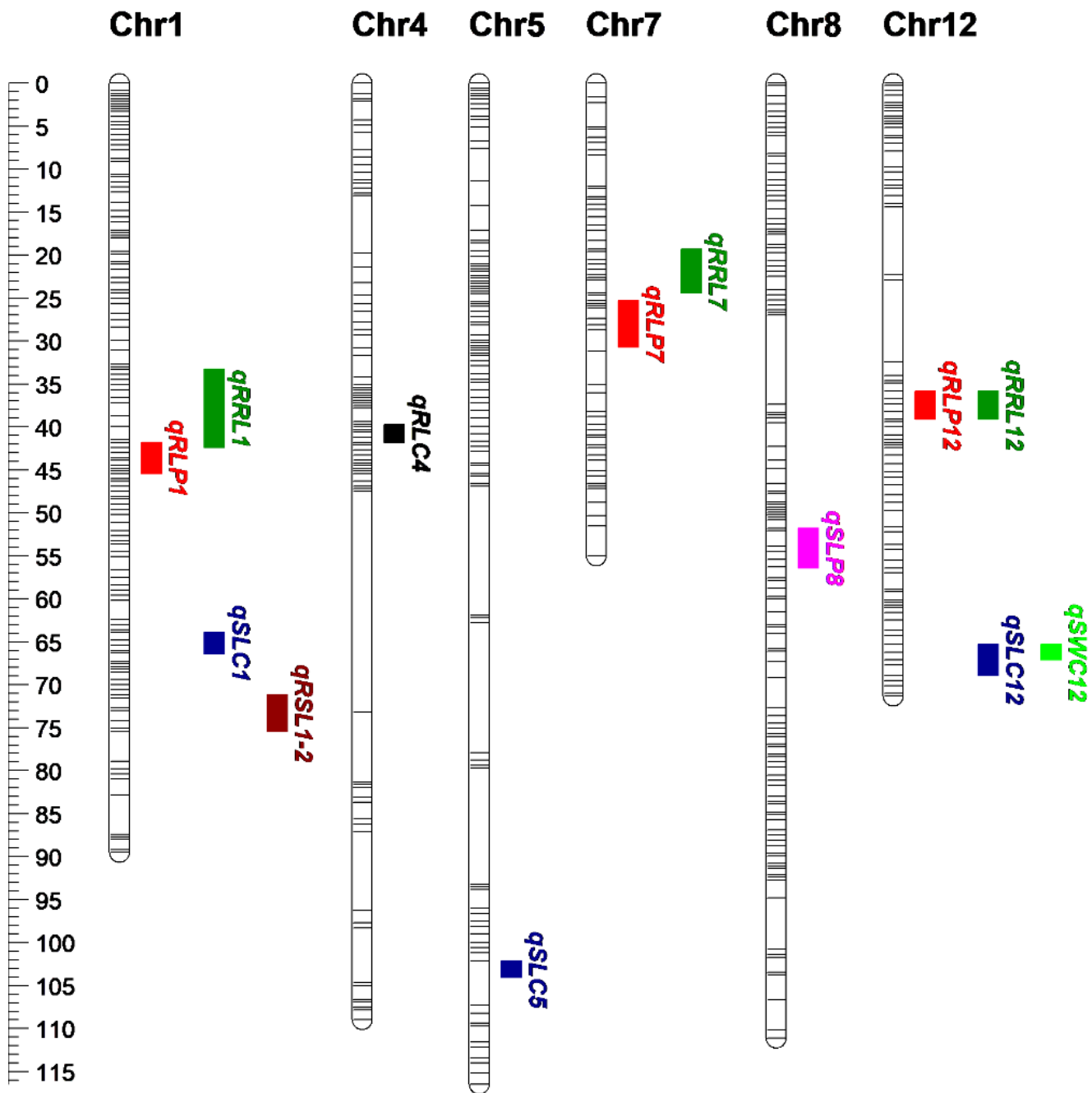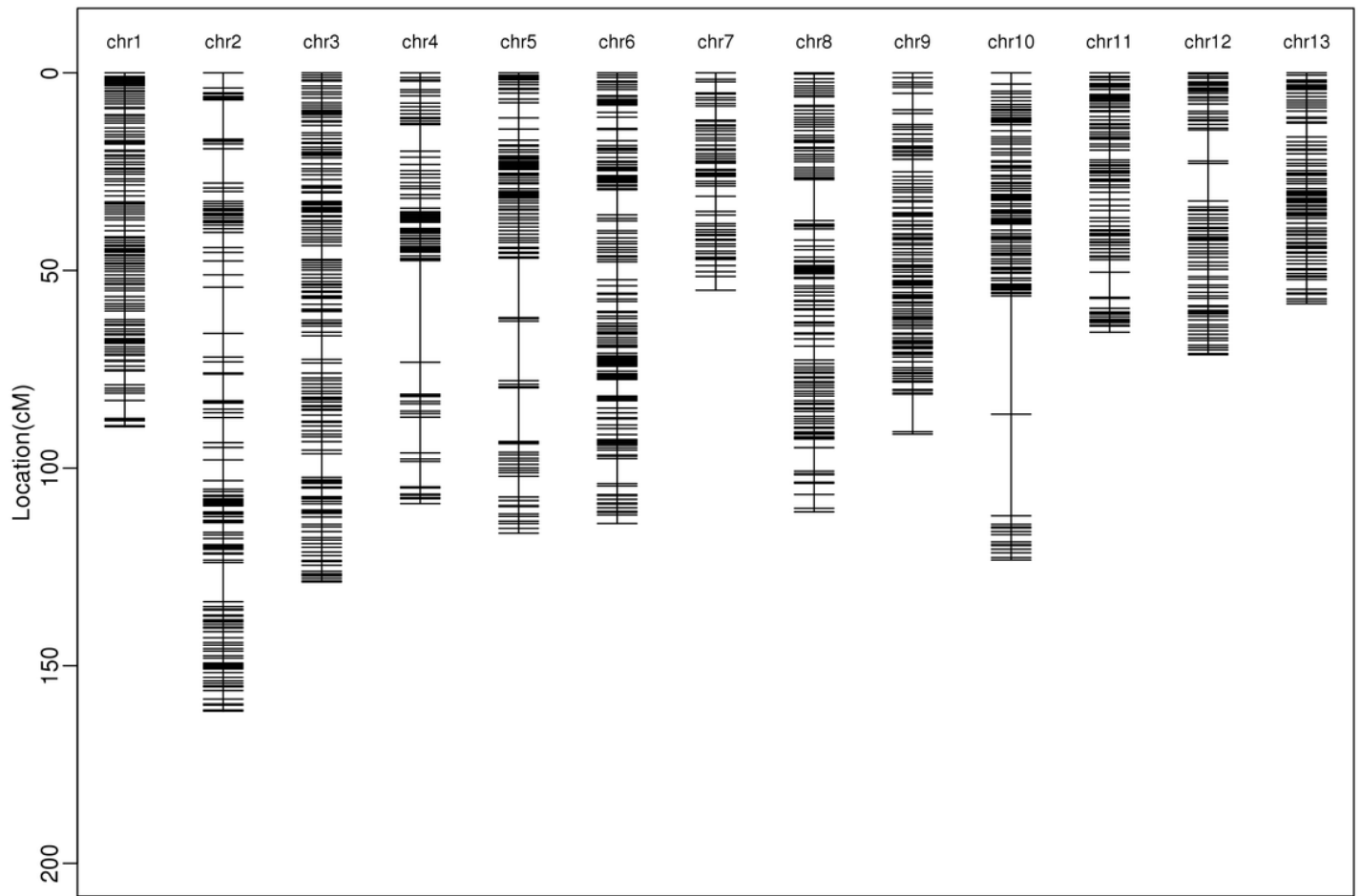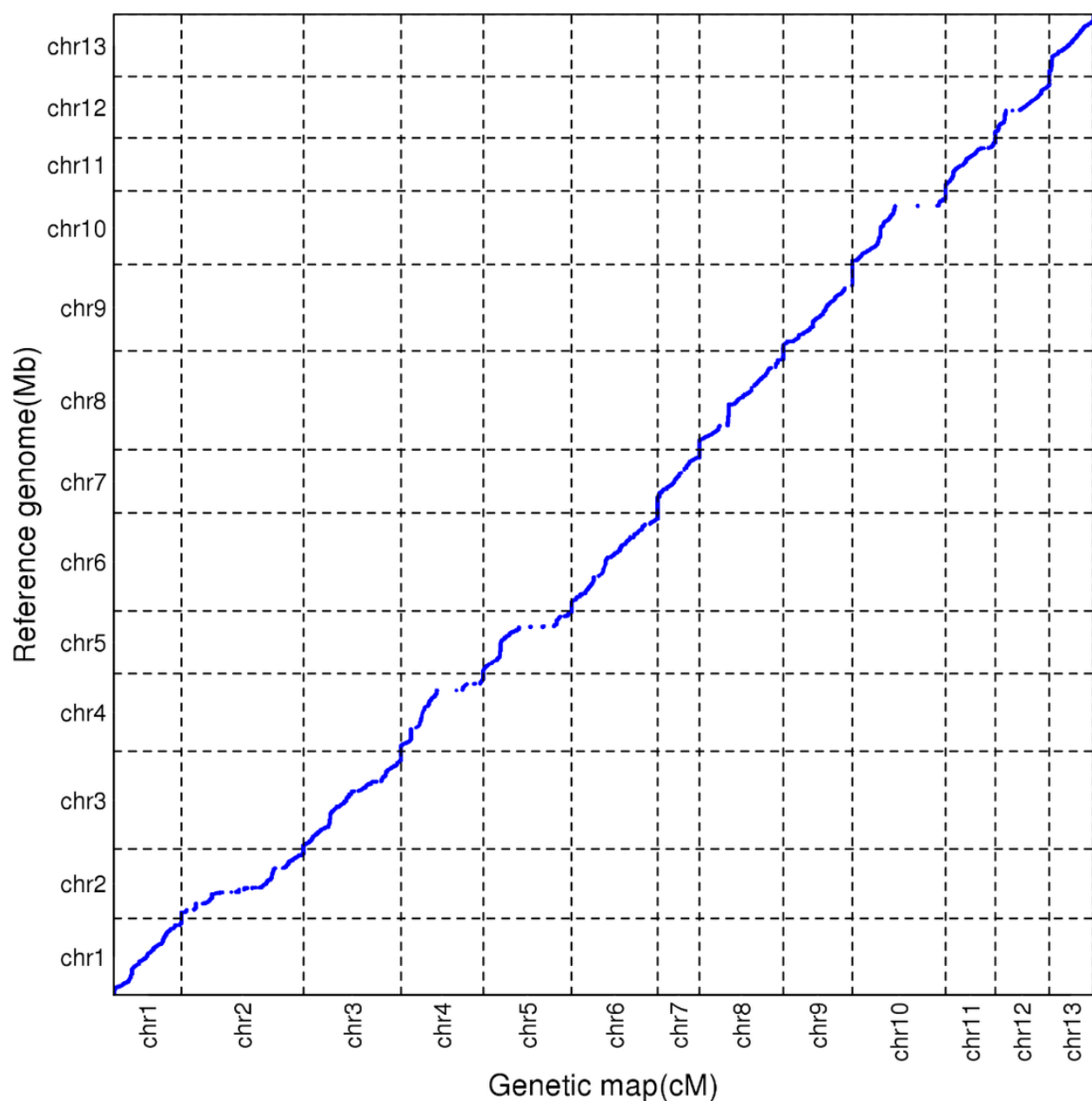Collinearity analysis of genetic map with the sesame reference genome Zhongzhi No. 13.

Figure 4

Frequency distributions of seedling weight (SW) (a, d), shoot length (SL) (b, e) and root length (RL) (c, f) under control and PEG stress condition in the RIL population.

Figure 5

Frequency distributions of relative SW (a, d), relative SL (b, e) and relative RL (c, f).

Figure 6

Chromosome location of identified stable QTLs in the RIL population. Genetic distances are shown at the left in cM. Bars in each chromosome represent bin-markers. Vertical bars of each QTL represent confidence intervals of 1-LOD and are located compared to the marker position on each chromosome.

Supplementary Files

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