Genetic basis analysis for indica germinability under low temperature using a new RILs population genotyping by whole-genome resequencing

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Abstract

Background : Improving the cold tolerance of rice at germination stage is an important objective to maintain rice yields. However, less analyses were carried out to detect the quantitative trait loci (QTLs) associated with cold tolerance using indica/indica population. Therefore, the genetic basis of cold tolerance of the indica varieties should be provided considerable attention

Results: In this study, a recombinant inbred lines (RILs) population comprising 126 lines derived from two widely used double-cropped indica rice varieties Wufeng B (WFB) and Changhui T025 (CHT025) was used to construct a high-density linkage map based on whole-genome resequencing. The high-density genetic map included 2,578 bins on 12 linkage groups and was 1762.80 cM in length, with an average interlocus distance of 0.68 cM. On the basis of newly constructed high-density genetic map, a total of 18 additive QTLs ranging from 34.55 to 315.21 kb on Nipponbare genome and two pairs of epistatic QTLs associated with cold stress at germination stage were detected, which indicated that the genetic basis of cold tolerance of WFB and CHT025 at germination stage is manly due to additive effects of several QTLs. Otherwise, the phylogenetic analysis showed that WFB is a typical indica variety while CHT025 is an interphyletic rice variety. Most of the favorable QTLs harbouring in indica WFB showed different chromosomal region from the QTLs associating with cold stress from japonica rice in previous studies, which indicated that indica might have different cold stress genetic mechanism comparing to japonica subspecies. Furthermore, we can incorporate these favorable QTLs existing in WFB into rice varieties to breed new cold tolerance indica male sterile maintenance line via marker-assisted selection; CHT025 is a better source of these cold tolerance favorable QTLs only only for the improvement of indcia but also for japonica restorer line germinability under low temperature via marker-assisted selection.

Conclusion : This population with high density genetic map will serve as better choice for identifying important quantitative traits of these two good indica germplasms, and these favorable QTLs exist in WFB and CHT025 can be used to breed new cold tolerance indica varieties via marker-assisted selection.

These authors have contributed equally to this work

Background

Rice, which evolved in tropical and subtropical areas, is sensitive to low temperatures during the whole life. Low temperature at the germination stage often causes delayed germination, decreased seedling vigor and low seedling establishment [1–3] while cold stress at the booting stage leads to male sterility in rice [4]. Therefore, improving the cold tolerance of rice is an important objective to maintain rice yields in rice breeding [5].

The Yangtze River valley is one of the most important rice production areas in China, early and late indica rice (double-cropped rice) are important crops in this region. In early indica rice season, rice varieties are always planted from late March to early April, and frequently harmed by exposure to low temperatures at the germination stage, resulting in large losses in rice production each year. Therefore, improving the rice cold tolerance at the germination stage in early indica rice season is one of the most important tasks for indica rice breeders to maintain rice production.

As a complex abiotic stress trait, the cold tolerance is genetically controlled by numerous quantitative trait loci (QTLs) and is also affected by the environment [6]. Over the past decades, QTL analysis underlying varietals difference for cold tolerance at different development stages in rice was conducted in a number of mapping populations, and the QTLs controlling cold tolerance have been roughly mapped to all the 12 rice chromosomes [7, 8–12]. Among these cold tolerance QTLs, some of them have been cloned [5, 13–18]. It is noticeable that most QTLs for cold tolerance detected by the populations deriving from crosses between indica and japonica cultivars, such as Nipponbare/9311 [19] and 02428/CH891 [6], because indica rice varieties are more sensitive to low temperatures, and suffer cold stress more frequently, than do varieties of the japonica subspecies [20–22]. Although japonica rice is generally more cold tolerant than indica rice, there might be favorable alleles for cold tolerance existing in indica that can be used to enhance cold tolerance [23, 24]. However, less analyses were carried out to detect the QTLs associated with cold tolerance using indica/indica population, especially for the double-cropped indica varieties. Consequently, little is known about the genetic information of germinate vigor after cold stress for indica rice. Therefore, the genetic basis of cold tolerance of the indica varieties should be provided considerable attention, and its gene or QTL conferring the indica cold tolerance trait should be located for further gene cloning and molecular breeding.

With the development of whole-genome re-sequencing, the construction of genetic maps has benefited from the development of SNPs that rely on automated sequencing and genotyping technologies [25, 26]. In rice, due to the the availability of the rice reference genome sequence (https://www.ncbi.nlm.nih.gov/assembly/GCF_001433935.1), it is possible to carry out high-throughput sequencing and provide an effective platform for direct identification of million of single nucleotide polymorphisms (SNPs) across the whole genome [6, 27].

In this study, a recombinant inbred lines (RILs) population was developed by using Wufeng B (WFB) and Changhui T025 (CHT025), where the two parents belonging to indica subspecies are widely used in the double-cropped rice area of China [28]. A high-density genetic linkage map of the RILs population was constructed by whole-genome re-sequencing approach. Furthermore, to evaluate the cold tolerance of indica rice at germination stage, we comprehensively investigated seedling length (SL) and root length (RL) for seven successive days after cold stress by the RILs population. The purpose was to detect major QTLs that could detect linkage markers for associate traits and could be applied to rice cold tolerance improvement, the QTLs detected and the selected RILs might be utilized to improve cold tolerance by the MAS approaches in future.

Methods

Plant materials

The RILs population comprised 126 lines, which were developed by single seed descent from a cross between Wufeng B (WFB) and Changhui T025 (CHT025). where WFB is an indica male sterile maintenance line, and CHT025 is an indica restorer line, both are wildly planted in double-rice cropping regions in south of china.

The two parents (WFB and CHT025) and other 254 rice varieties were used to analyze the genetic distances. These rice germplasms included indica varieties (such as ZhengShan 97, Zaoxian 45), japonica varieties (such as Longgeng 31, C418) and landraces (such as Maguzi, Jiangtang1) and collected from more than 12 provinces in China (Supplemantary table sheet 1). The collection sites and other information on each rice accession is available in a database at http://www.chinariceinfo.com/

Genomic DNA extraction, sequencing and SNP discovery
Genomic DNA was extracted from each individual RILs and the two parents by CTAB method. Each DNA was cut by physical method and the adapters were ligated to barcode the DNA of each line. DNA fragments were ~ 300 bp in length was selected by gel electrophoresis, and the libraries were enriched by PCR amplification. The resulting libraries were sequenced in the MGISEQ-2000 instrument owned by The Beijing Genomics Institute. The raw reads were then filtered and sorted according to indices and the RawSNPs were filtered out by alignment with Nipponbare reference genome by BWA and GATK tools. To obtain high-confidence SNP markers for genetic map construction, all RawSNPs identified between the parental lines were filtered as follows: depth at each SNP in both parental lines > 10, mapping quality of reads > 20. We further selected SNPs which were 100% homozygous alleles in each parental line. The final candidate SNPs were used for genotyping of the RILs population. For genotype calling, reads of RILs individuals were filtered as follows: read depth > 10, mapping quality of reads > 20. SNPs that had > 50% missing or ambiguous data were excluded. At last, the high-confidence SNPs was obtained for genetic map construction.
BinMap construction
The construction of high-density genetic map was performed as described by Jiang et al. [6]. In brief, consecutive 100-kb intervals that lack a recombination event in the population were combined into bins via sliding-window approach [25]; then, the bin genetic map was constructed using JoinMap® 4.1 software [29].
The genetic distances analysis for the two parents
The 56K SNP chip [30] was applied in SNP genotyping for the two parents (WFB and CHT025) and other 254 rice varieties. The model-baded program Popgene [25] was used to infer population structure and to assign individual varieties into subpopulation.
Evaluation of seedlings performance after cold stress
The 126 RILs together with the parents WFB and CHT025 were used to evaluate the cold tolerance. Each sample with fifteen to eighteen vigorous seeds was placed in a Petri dish containing tap water and incubated at 25 °C to achieve the effect of germination, germination was determined by the emergence of the radicle or/and the plumule. Then the seedlings were subjected to low temperature treatment (15 °C, day/15 °C, night) in the chamber for 7 days, after which the plants were returned to normal temperatures (28 °C, day/28 °C, night) to begin the recovery process. All the experiments were conducted in a standard growth chamber. Ten seedlings with uniform performance for each sample were strictly collected to measure recovery ability, including root length (RL) and shoot length (SL) in the subsequently 7 days.
The temperature treatment was repeated three times in the standard growth chamber, and mean values of three replicates were used for data analysis.
Statistical and genetic analyses
The difference between the two parents and the progeny for each trait was detected, the means, StdError, Skewness and Kurtosis were carried out by ICIMapping 4.2 software (http://www.isbreeding.net)
Identification of QTLs
Single-trait analyses for each day separately were detected based on the inclusive composite interval mapping (ICIM) of ICIMapping 4.2 (http://www.isbreeding.net), threshold LOD of 2.5 was applied [6]. The location genetic effects, and phenotypic variation explained of QTL were determined according to the peak value of QTL interval. QTL nomenclature was followed by the method of McCouch et al. [32].

Results

The kinship analysis of WFB and CHT025

Power marker and Popgene was used to analyze the panel structure. According to the genetic distance analysis, the structure analysis identified a subpopulation (99 accessions), and a second group (157 accessions) constituted by varieties derived from two major subspecies (Fig. 1). From the population structure analysis, we found that WFB belong to the first group and is a typical indica rice variety while CHT0125 belong to the second group, but CHT025 showed a far distance from the typical japonica, such as Longgeng 31. Thus, we concluded that CHT025 is a interphyletic rice variety possessing some japonica gene fragments in indica genetic background.

Linkage maps in the RILs population

The high-quality sequences were used for identification of SNPs. A total of 5,624,129 putative SNPs were determined in all the 128 population level (two parents and 126 RILs individuals). After the strict filtering, 701,310 (12.47%) SNP loci were retained to construct the genetic map in the final (Table 1). Of the 701,310 SNP loci, sliding-window approach to transfer bins markers and a total of 2,578 evenly distributed genomewide polymorphic bins were used to construct linkage maps in the RILs population.

Table 1
The raw and filtrated SNPs number information of the RILs population

ChrName	RawSNPNum	DiffPSNPNum
chr01	582664	81931
chr02	514404	54417
chr03	477866	73768
chr04	512878	87008
chr05	397048	55243
chr06	451377	73301
chr07	433395	30570
chr08	451779	19386
chr09	356458	71559
chr10	390722	38377
chr11	564030	43633
chr12	491508	72117
Total	5624129	701310

Based on these bin markers, we developed a high density genetic linkage map, with a total of 2,578 bins spanning 1,762.80 cM (Table 2; Fig. 2). The genetic distance between adjacent bins ranging from 0.01 to 7.24 cM, with an average interval of 0.68 cM.

Table 2
The bin makers number and distance for each chromosome of the RILs population

ChrName	MarkerNum	GeneticDistance (cM)	AvergaeDistance (cM)
chr01	303	185.47	0.61
chr02	285	196.02	0.69
chr03	235	177.75	0.76
chr04	240	160.43	0.67
chr05	177	152.21	0.86
chr06	226	132.68	0.59
chr07	180	125.80	0.70
chr08	159	145.77	0.92
chr09	171	123.05	0.72
chr10	166	106.44	0.64
chr11	208	125.46	0.60
chr12	228	131.73	0.58
Total	2578	1762.80	0.68

Cold tolerance performance of the two parental lines and the RILs population

After cold stress for the germinated seeds, the SL and RL of WFB and CHT025 increased rapidly under normal temperature condition. Significant differences were observed in SL after cold stress between WFB and CHT025 from 1st day to 6th day after cold stress (ACS), and WFB showed longer SL that these of CHT025. At the 7th day ACS, the SL of WFB and CHT025 are almost same. For RL, WFB showed a higher values than these of CHT025 from 1st day to 7th day ACS (Table 3; Fig. 3).

Table 3
Performance of parents and RILs population for shoot length and root length after cold stress (ACS)

TraitName	CHT025	WFB	Minimum	Maximum	Mean	StdError	Skewness	Kurtosis
Shoot length ACS								
SL1*	0.66	1.39	0.50	2.69	1.19	0.52	0.93	-0.13
SL2	1.65	3.09	1.06	3.10	2.36	0.40	-0.68	0.30
SL3	2.80	3.98	1.63	4.28	3.18	0.52	-0.66	0.15
SL4	3.40	4.68	2.32	5.41	3.94	0.61	-0.25	-0.13
SL5	3.84	5.04	2.57	6.92	4.64	0.81	-0.07	-0.17
SL6	4.79	5.21	2.93	7.83	5.30	1.11	-0.03	-0.61
SL7	6.13	6.03	3.09	8.65	6.01	1.18	-0.11	-0.42
Root length ACS								
RL1	1.23	2.50	0.53	5.78	2.16	1.19	1.02	0.32
RL2	3.19	4.01	1.01	6.19	3.60	1.01	-0.08	0.13
RL3	4.07	4.55	1.17	6.76	4.07	1.11	-0.14	0.07
RL4	4.30	4.77	1.46	7.42	4.62	1.13	-0.39	0.31
RL5	4.43	4.99	2.06	7.96	5.01	1.13	-0.27	0.21
RL6	4.50	5.18	1.83	7.95	5.14	1.11	-0.39	0.51
RL7	5.83	6.82	2.29	8.85	5.21	1.22	0.03	-0.10
* SL1: the shoot length for the first day after cold stress, RL1: the root length for the first day after cold stress, and so on; the same below.								

The phenotypic values of the RL and SL within the RILs population were presented in Fig. 4. The length of the root and shoot of the RILs population segregated continuously and both skewness and kurtosis values were less than 1.0 at most of the measuring stages (Table 3). It appeared that segregation of the length of the root and shoot within the RILs population was normally distributed and suitable for QTL analysis. Transgressive segregants were also observed at all measuring stages for both SL and RL (Fig. 4).

Cold tolerance QTLs for SL

QTLs analysis linked to molecular markers by QTL IciMapping v4.2 Mapping software was performed for SL in this study. A total of nine significant QTLs were identified on four of the 12 chromosomes and the respective alleles explain 6.86–15.73% of the total phenotypic variation (Table 4; Fig. 5). No QTLs were present on chromosomes 1, 5, 6, 7, 8, 9, 11 or 12. At these loci, the alleles with increasing SL were from WFB for qSL2.1, qSL2.2, qSL2.3, qSL2.4, qSL4.1, qSL4.2, qSL10.1 and qSL10.2, and this with increasing SL from CHT025 for qSL3.1. Of these cold tolerance QTLs for SL, only two QTLs, qSL3.1 and qSL4.2, were expressed in different days ACS. No additive QTLs associated was detected on the first day ACS. The physical distance for these additive QTLs was 49.78-315.21 kb on Nipponbare genome (Table 4; Supplemantary table sheet 2). Otherwise, one pair of QTLs qSL8.1 and qSL8.2, showing epistatic interaction (additive × additive) was detected on 7th day ACS, which explained 23.43% of the variance (Table 5; Fig. 6).

Table 4
Putative QTLs associated with shoot length after cold stress in the RILs population

QTL	Chromosome	LeftMarker	RightMarker	LOD	PVE(%)	Add	Date	PositiveParent	Physical distance (kb)
qSL2.1	2	chr02_bin331	chr02_bin332	3.37	12.10	-0.13	SL2	WFB	49.78
qSL2.2	2	chr02_bin568	chr02_bin569	4.09	13.48	-0.45	SL7	WFB	57.19
qSL2.3	2	chr02_bin577	chr02_bin578	2.75	8.27	-0.24	SL5	WFB	136.08
qSL2.4	2	chr02_bin589	chr02_bin592	5.57	15.73	-0.23	SL4	WFB	315.21
qSL3.1	3	chr03_bin838	chr03_bin839	3.00	8.34	0.34	SL6	CHT025	931.17
				2.56	6.86	0.15	SL4		
qSL4.1	4	chr04_bin1095	chr04_bin1093	4.61	13.25	-0.43	SL6	WFB	200.03
qSL4.2	4	chr04_bin1093	chr04_bin1096	3.37	9.51	-0.18	SL4	WFB	299.33
				3.30	9.86	-0.25	SL5		
qSL10.1	10	chr10_bin2080	chr10_bin2081	3.87	11.34	-0.27	SL5	WFB	88.96
qSL10.2	10	chr10_bin2101	chr10_bin2100	3.77	10.22	-0.17	SL3	WFB	151.57

Table 5
Putative epistatic QTLs associated with shoot length after cold stress in the RILs population

QTL	LeftMarker	RightMarke	QTL	LeftMarker	RightMarke	LOD	PVE(%)	Add1	Add2	AddbyAdd	Date
qSL8.1	chr08_bin1744	chr08_bin1745	qSL8.2	chr08_bin1755	chr08_bin1756	5.23	23.43	-0.12	0.07	0.53	SL7

Cold tolerance QTLs for RL

A total of nine QTLs that significantly influenced RL were identified on chromosomes 1, 2, 5 and 12 (Table 6; Fig. 7). One QTL, qRL12, were constantly detected from 1st to 3rd day ACS, two QTLs, qRL2.2 and qRL2.3, were detected in two days ACS, while five QTLs, qRL1.1, qRL1.2, qRL1.3, qRL2.1, qRL5.1 and RL5.2, were just detected one day ACS. At these loci, the alleles associated with increasing RL were from WFB for qRL1.1, qRL1.2, qRL2.2, qRL2.3 and qRL12, and those with increasing RL from CHT025 for qRL1.3, qRL2.1, qRL5.1 and RL5.2. The physical distance for these additive QTLs was 34.55-200.01 kb on Nipponbare genome (Table 6; Supplemantary Table 2). Epistatic interaction between qRL8.1 and qRL8.2 (additive × additive) explained 25.05% of the variance was detected on 1st day ACS (Table 7; Fig. 8).

Table 6
Putative QTLs associated with root length after cold stress in the RILs population

QTL	Chromosome	LeftMarker	RightMarker	LOD	PVE(%)	Add	Date	PositiveParent	Physical distance (kb)
qRL1.1	1	chr01_bin21	chr01_bin22	4.87	11.09	-0.41	RL3	WFB	66.27
qRL1.2	1	chr01_bin47	chr01_bin48	3.59	14.66	-0.43	RL7	WFB	117.92
qRL1.3	1	chr01_bin234	chr01_bin235	2.78	6.06	0.29	RL3	CHT025	76.92
qRL2.1	2	chr02_bin535	chr02_bin536	3.44	7.34	0.38	RL4	CHT025	109.12
qRL2.2	2	chr02_bin579	chr02_bin580	4.00	16.48	-0.43	RL6	WFB	34.55
				2.60	10.67	-0.36	RL7		
qRL2.3	2	chr02_bin581	chr02_bin582	7.14	15.42	-0.56	RL4	WFB	101.50
				4.02	13.39	-0.43	RL5		
qRL5.1	5	chr05_bin1135	chr05_bin1136	2.53	8.27	0.33	RL5	CHT025	200.01
qRL5.2	5	chr05_bin1136	chr05_bin1137	3.24	7.16	0.32	RL3	CHT025	100.47
qRL12	12	chr12_bin2665	chr12_bin2666	3.24	11.18	-0.42	RL1	WFB	93.06
				2.66	9.74	-0.31	RL2		
				5.44	12.56	-0.42	RL3		

Table 7
Putative epistatic QTLs associated with root length after cold stress in the RILs population

QTL	Chromosome	LeftMarker	RightMarker	QTL	Chromosome	LeftMarker	RightMarker	LOD	PVE(%)	Add1	A
qRL8.1	8	chr08_bin1749	chr08_bin1750	qRL8.2	8	chr08_bin1754	chr08_bin1755	5.15	25.05	0.03	0

Discussion

The potential use of WFB/CHT025 RILs population

It is well known that a high quality linkage map make it feasible to increase QTL solution [6]. However, it is difficulty to identify enough molecular markers for genetic map construction by traditional marker develop technology, such as SSRs and RFLP [33]. With the development of genome sequence technology, SNP markers have been recognized as important candidate markers due to their high abundance and relatively even distribution across the genome [26]. In this study, a RILs population consisting of 126 individuals derived from CHT025/WFB was developed, and 1,257 bins makers have be developed to construct a high density genetic map. Compared with traditional genetic map constructed by crosses of indica/indica, the average interval size between adjacent bins makers of WFB/CHT025 RILs population was only 0.68 cM, which can effectively meet the requirements of the high QTL mapping resolution. Moreover, the high density map also provides a genetic framework for the rice reference genome assembly.

The two parents, WFB and CHT025, are widely planted in double-rice cropping regions in south of china and also used as important rice germplasms to breed new indica varieties, such as indica male sterile maintenance and restorer lines [28]. That means there might be some favorable alleles existing in WFB and CHT025, thus, it is necessary to extract the favorable genes (QTLs) harbouring in the WFB and CHT025. This population with high density genetic map will serve as better choice for identifying important quantitative traits of these two good indica germplasms, and provide a chance to develop makers linked to favorable QTLs of WFB and CHT025 for future indica molecular breeding programmes.

Genetic basis for indica cold tolerance

Indica varieties are widely grown in double-rice cropping regions in subtropical zones, and is frequently harmed by exposure to low temperatures at the germination stage. Due to the lower cold tolerance than japonica, the studies of modern indica cultivated rices for cold tolerance are limited [24]. In our study, two double-cropped indica rice varieties CHT025 and WFB were used to address the genetic basis of cold tolerance for the first time. The cold performances showed that SL and RL of WFB after cold stress exhibits higher values than those of CHT025, which implied that WFB has a higher recovery ability after cold stress at germination stage than that of CHT025, and confirmed that different indica varieties show different performance after cold stress [24].

The genetic control analysis for CHT025 and WFB using the RILs, which can reflect the the genetic effect such as additive and epistatic effects, showed that there are a total of 18 additive QTLs and only two pairs of QTLs showing epistatic interactions associated with SL and RL ACS, which indicates that the genetic basis of strong cold tolerance of WFB and CHT025 at germination stage is manly due to additive effects of several QTLs. Transgressive segregation was observed in the frequency distribution of SL and RL of the RILs population, which implied that there were positive alleles from WFB and CHT025, consequently, the QTLs with favorable alleles from WFB and the QTLs from CHT025 were all detected. For these QTLs, most of them possessing WFB positive alleles increased SL and RL ACS, which implied that the cold resistant indica varieties might possess more favorable genes than the cold sensitive ones. Otherwise, many of the indica cold tolerance QTLs varied different in different day ACS, indicating that cold tolerance in indica rice at different stages might be related to different genetic mechanisms, the result is according with the conclusion of previous studies [34, 35]. Thus, to cope with cold stress at a given stage, the rice variety must be introgressed with a certain number of QTLs to accumulate enough cold tolerance.

Zhang et al. [24] showed that many cold-tolerant QTLs of indica varieties might come from japonica varieties during the process of rice cross breeding. But the phylogenetic analysis showed that WFB is a typical indica variety and possess some favorable QTLs (genes) conferring cold tolerance. The result indicated that the indica varietes might have their own cold tolerant genes. Furthermore, most of the favorable QTLs of our study showed different chromosomal region from the QTLs associating with cold stress derived from japonica rice in previous studies, which indicated that indica might have different genetic mechanism for cold stress comparing to japonica subspecies.

Relationship of mapped QTLs and previously identified genes

Comparison of QTL positions on the rice genome with those detected in prior studies provided information about the QTL consistency despite the differences in the genetic materials, marker types, and screening methodology. We found that qSL2.1 on chromosome 2 overlaps with qESCT-2, qSL3.1 on chromosome 3 is near qESCT-3 and qRL12 on chromosome 12 is near qESCT-12 [11]. For the above QTLs, where the indica rice Katy alleles underlying them showed negative additive effects and decreased early-seedling cold tolerance [11]. The chromosome 12 locus qRL12 also maps to an adjacent location as does qCTS-12 [35], the indica rice‘Hua-jing-xian 74 allele underlying qCTS-12 showed negative additive effects and decreased cold tolerance at the seedling stage. The chromosome 5 loci qRL5.1 and qRL5.2 overlaps with qLTG-5-1, where the indica N22 decreased low temperature germination [21]. qSL10.1 maps to an adjacent location as does qLTG10.2, the allele of qLTG10.2 from indica rice XQZB increased germinate more rapidly than the wild rice [19]. The results indicated that all the above five QTLs associating with cold stress might exist in many modern indica rice varieties, therefore, we should pay more attention to these QTLs in indica rice breeding program in future. Otherwise, the other 12 OTLs are reported here for the first time. For all the QTLs, they all were located on small genomic regions (34.55 to 315.21 kb on Nipponbare genome). This result provides a springboard for map-based cloning of these QTL and is helpful in understanding the mechanism of seed recovery under low-temperature conditions.

Using of the cold tolerance QTLs detected by WFB/CHT025 RILs population

Typically, japonica rice exhibits better cold tolerance than indica rice, and many QTLs that confer cold tolerance at different stages from japonica rice cultivars have been identified. Therefore, it is suggested that the cold tolerance of indica rice can be improved using genes from japonica rice through molecular breeding. However, it is not so easy to transfer the japonica cold tolerance QTLs (genes) to indica genetic background by molecular breeding, because there exists partial incompatibility between subspecies [11]. If there are favorable QTLs (genes) for cold tolerance in indica, we can transfer these tolerance QTLs (genes) to other indica genetic background to improve indica cold tolerance through molecular breeding.

Recently, some studies have began to study indica cold tolerance and identify several favorable cold tolerance QTLs (genes) hidden in the indica germplasm [23, 24]. Our QTLs analysis confirmed that there are actually some favorable QTLs (genes) for cold tolerant in modern indica variety. More importantly, these favorable cold tolerance QTLs (genes) were detected in two widely indica rice varieties, therefore, it is feasible to pyramid these favorable cold tolerance QTLs to breed new indica variety. For example, taking WFB as the parental material, we can incorporate these favorable QTLs exist in WFB into rice varieties to breed new cold tolerance indica male sterile maintenance line via marker-assisted selection; CHT025 is a better source of favorable QTLs of SL and RL not only for the improvement of indcia but also for japonica restorer line germinability under low temperature, because it is an interphyletic rice variety.

Conclusions

To evaluate the cold tolerance of indica rice at germination stage, a recombinant inbred lines (RILs) population with high-density genetic map derived from two widely used double-cropped indica rice varieties was developed. This high density genetic map will serve as better choice for identifying important quantitative traits of these two good indica germplasms. On the basis of newly constructed high-density genetic map, a total of 18 additive QTLs ranging from 34.55 to 315.21 kb on Nipponbare genome and two pairs of epistatic QTLs associated with cold stress at germination stage were detected. Most of the favorable QTLs harbouring in indica WFB showed different chromosomal region from the QTLs associating with cold stress from japonica rice in previous studies, which indicated that indica might have different cold stress genetic mechanism comparing to japonica subspecies. These favorable QTLs exist in WFB and CHT025 can be used to breed new cold tolerance indica varieties via marker-assisted selection.

Abbreviations

QTLs
Quantitative trait loci;
RILs
Recombinant inbred lines;
WFB
Wufeng B;
CHT025
Changhui T025;
SNPs
Single nucleotide polymorphisms;
SL
Seedling length;
RL
Root length;
ACSL
After cold stress

Declarations

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Availability of data and materials
All data generated or analysed during this study are included in this published article and its supplementary information files.

Competing interests
The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Authors' contributions

JB and HH designed the research work, annotated the data and drafted the manuscript. PW, GW, XL, RL, AZ performed the experiments. YC, DZ, JT, ST, XZ, CL, CL, YW, QC developed the population; and all Authors read and approved the manuscript.

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

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Figures

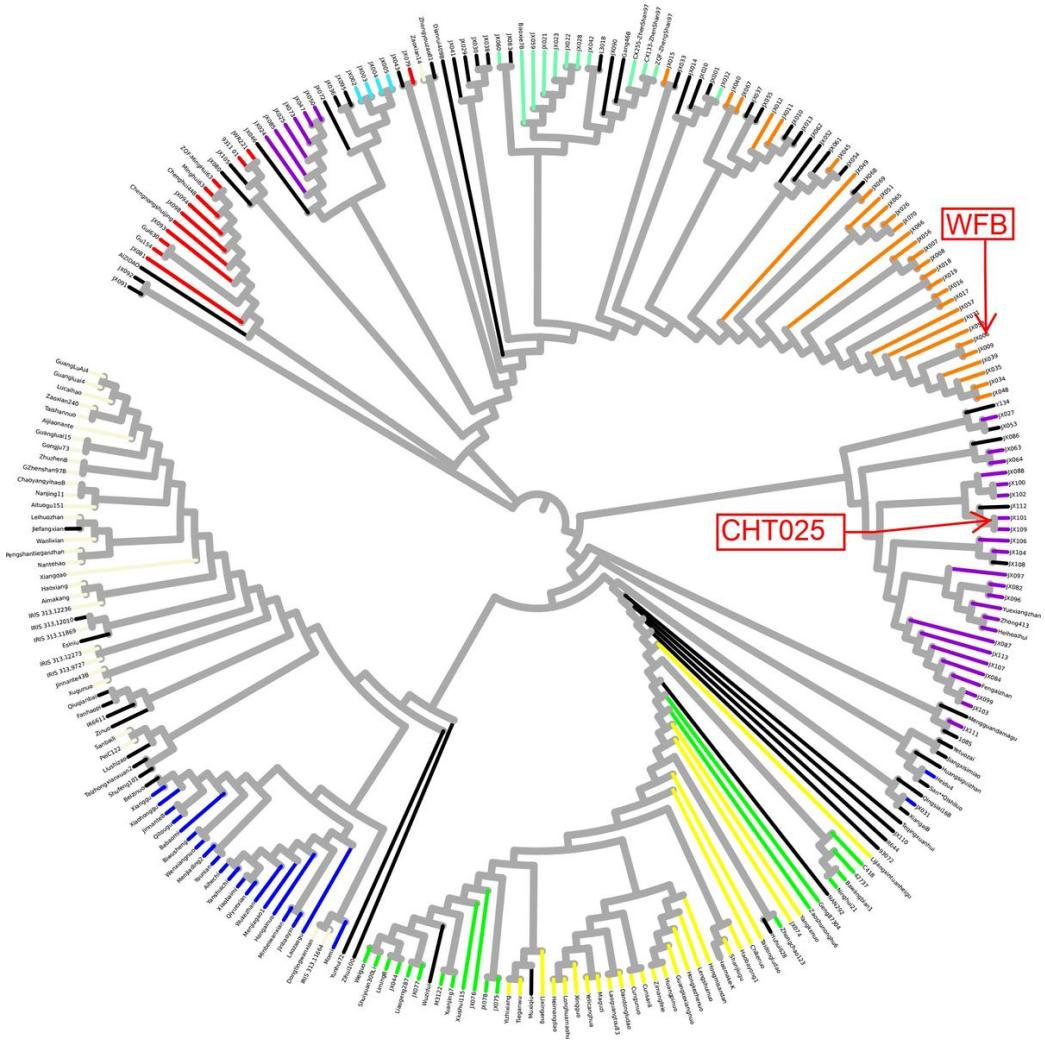

Figure 1

Population structure phylogenetic tree

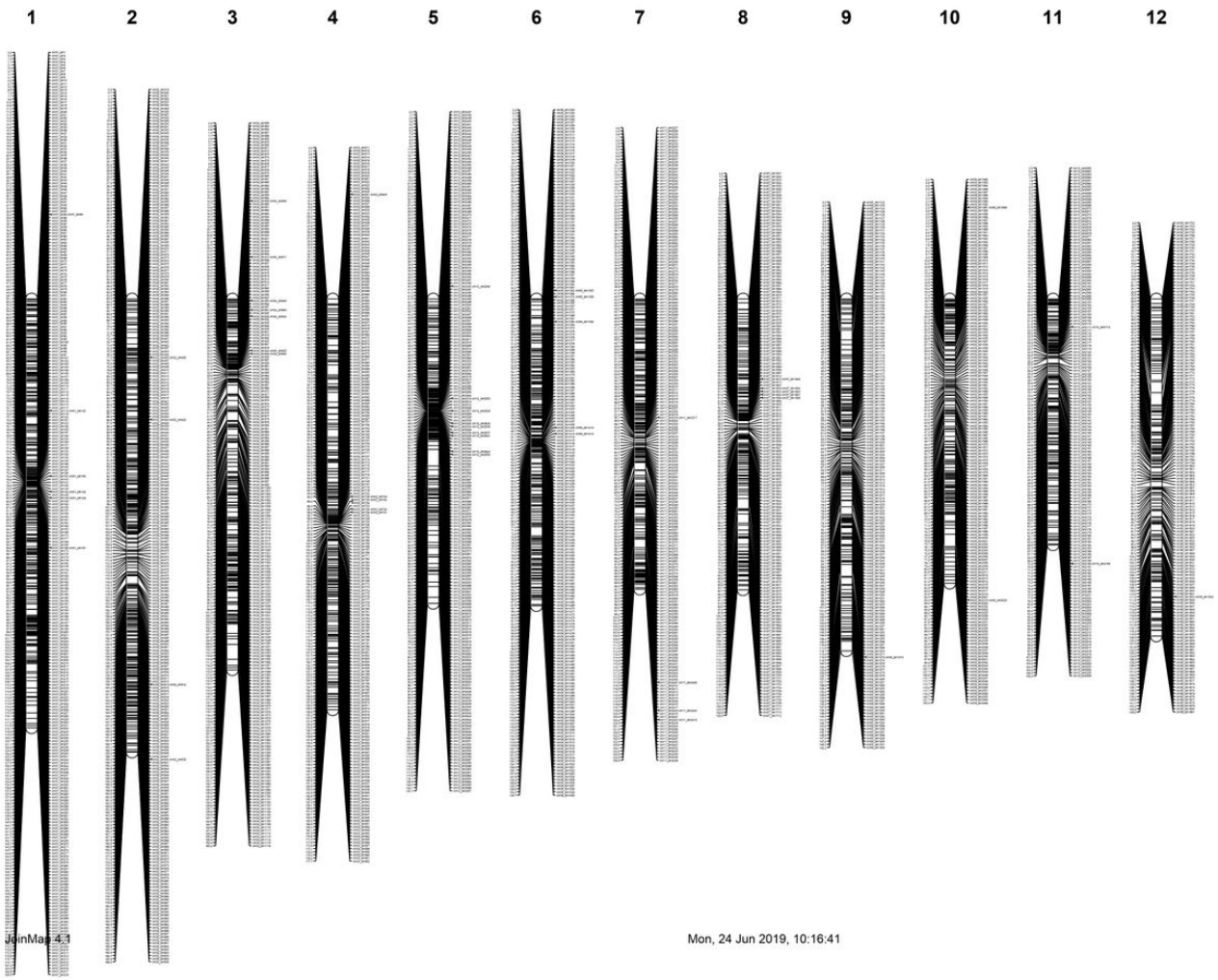

Figure 2

The high density linkage group for the RILs population

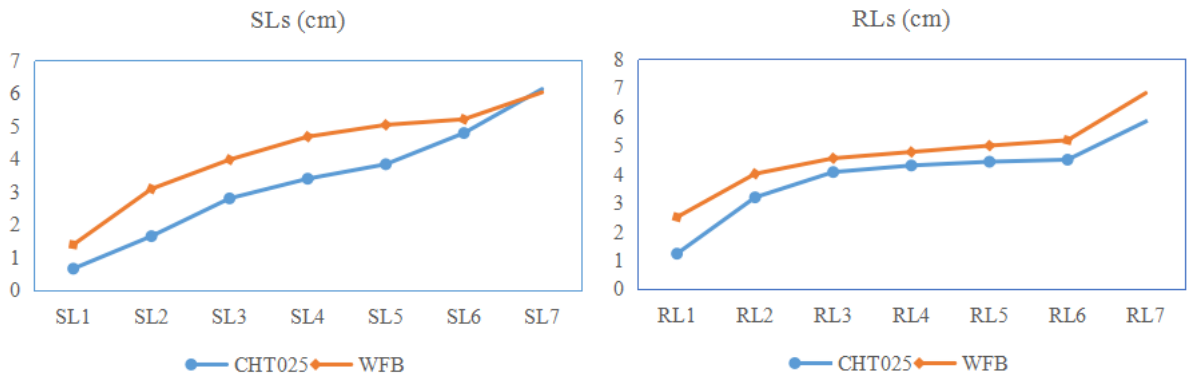

Figure 3

Performance of parents for shoot length and root length at continuous seven days after cold stress SL1: the shoot length for the first day after cold stress; RL1: the root length for the first day after cold stress; and so on.

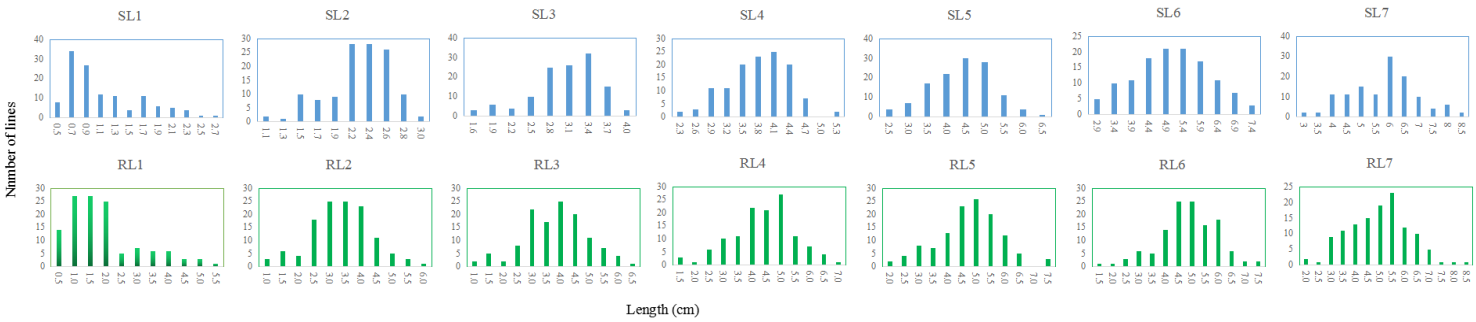

Figure 4

Frequency distribution of shoot length and root length at continuous seven days after cold stress in RILs population

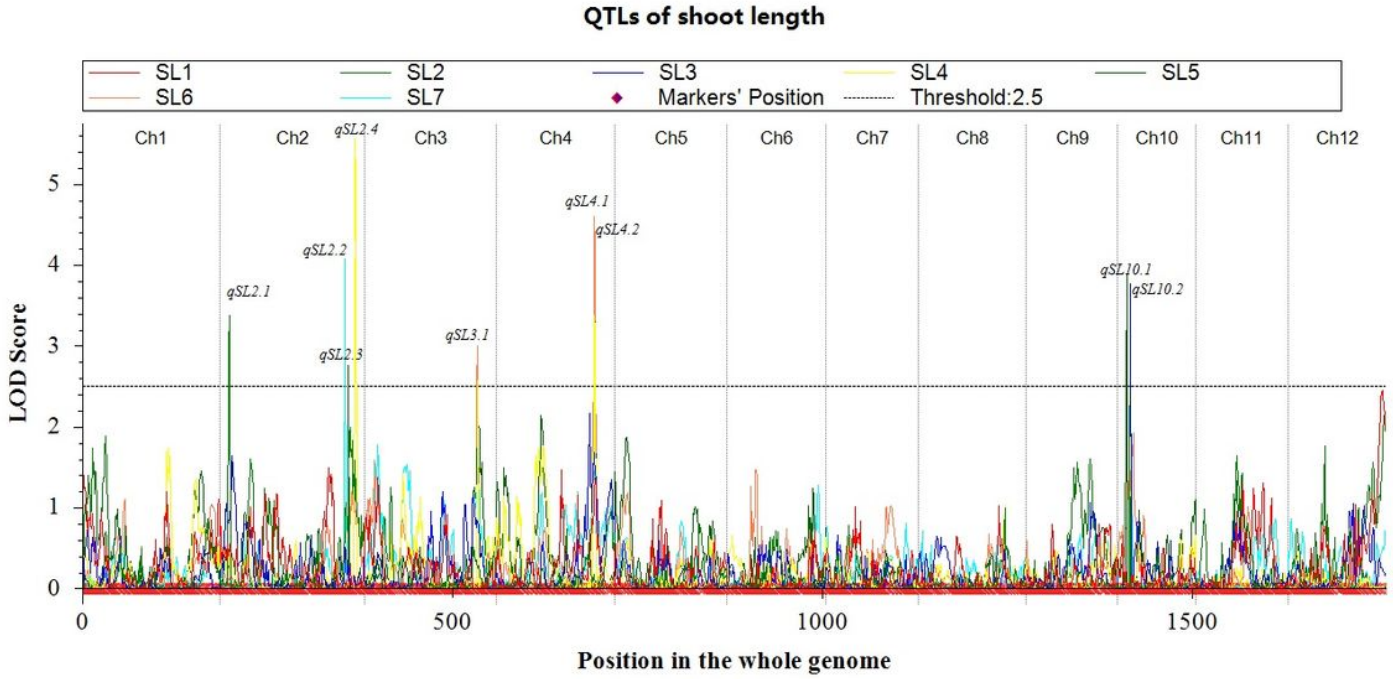

Figure 5

The QTLs position for shoot length after cold stress in RILs population

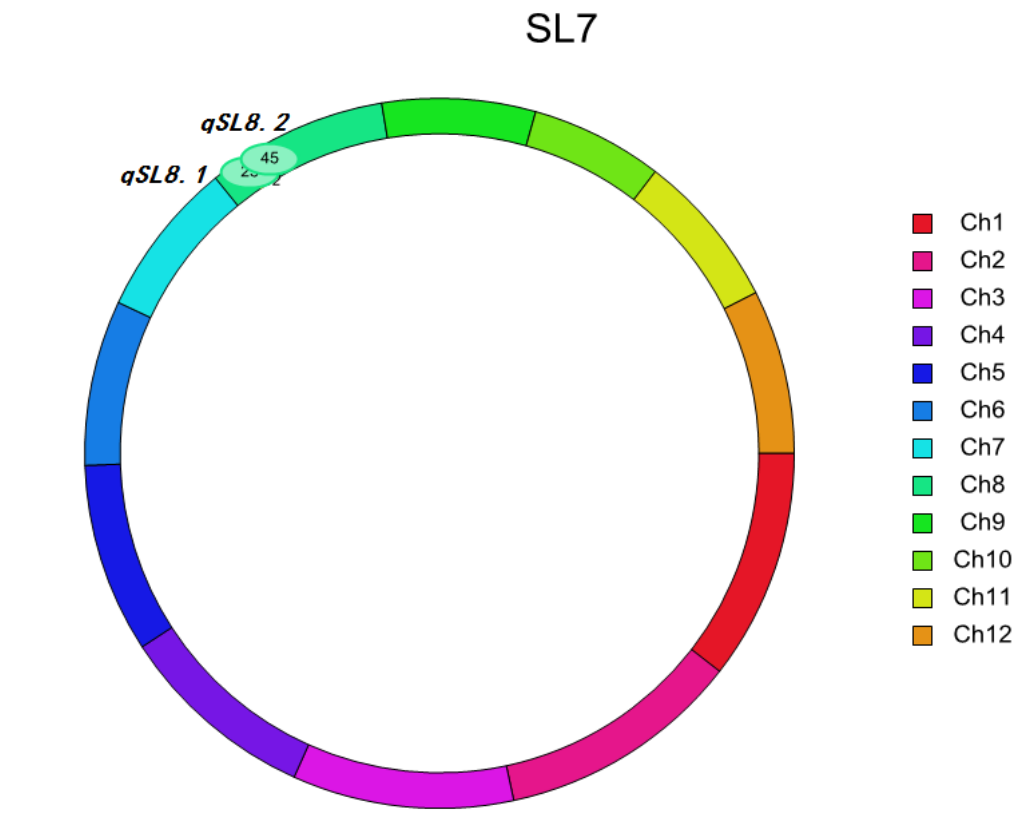

Figure 6

Putative epistatic QTLs position for shoot length after cold stress in the RILs population

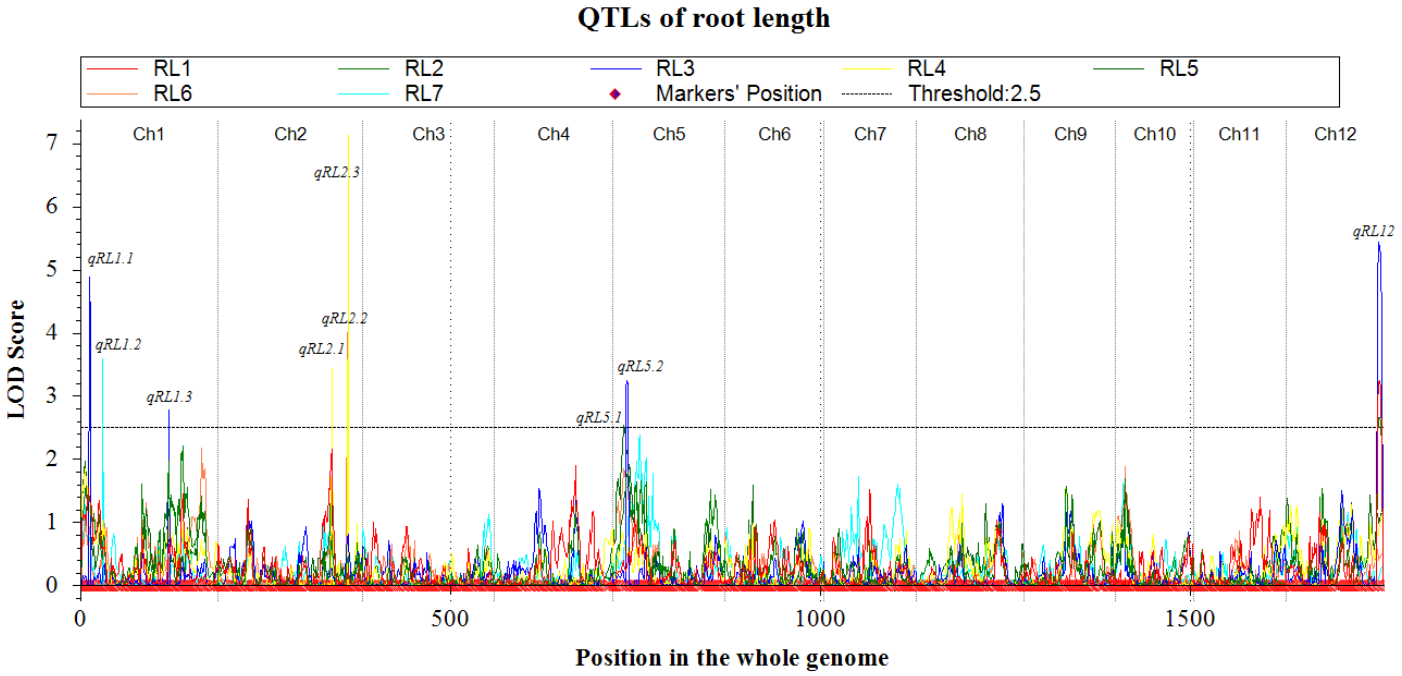

Figure 7

The QTLs position for root length after cold stress in RILs population

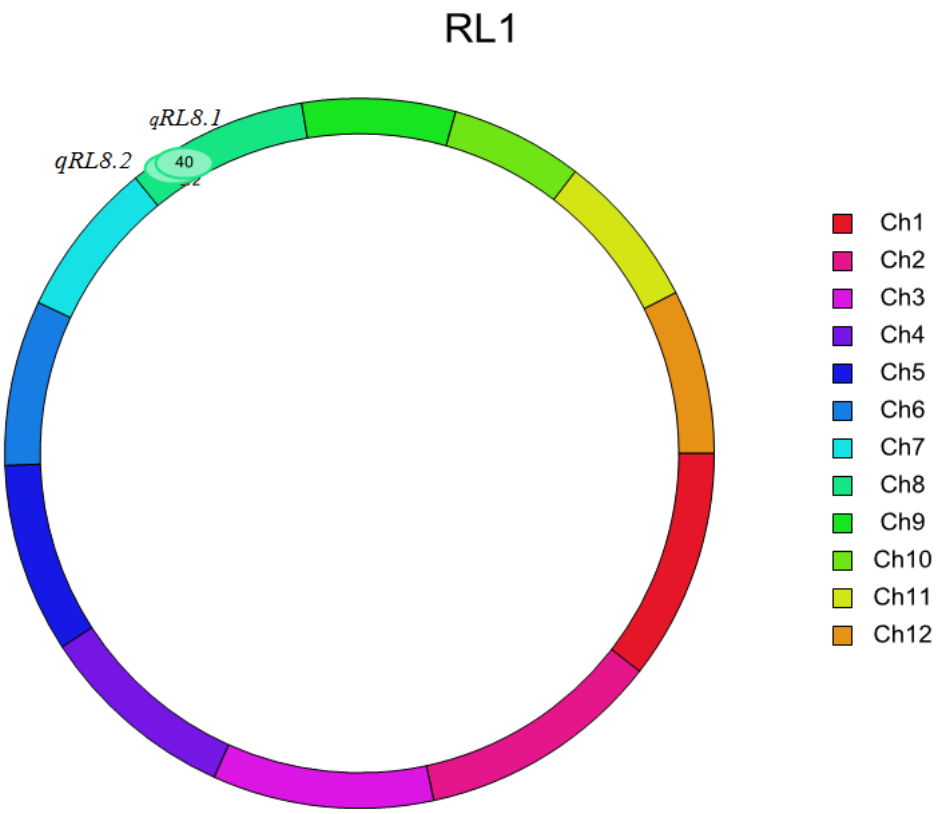

Figure 8

Putative epistatic QTLs position for root length after cold stress in the RILs population

Supplementary Files

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- Supplemantarytable.xls