QTLs Detection for Low-Temperature Germination in Rapeseed by QTL-seq and Linkage Mapping Approach

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Abstract

Rapeseed is a major oil crop in the world, which is easily affected by low-temperature stress. Low-temperature delays seed germination and increases seedling mortality that adversely affects rapeseed growth and production. To understand the genetic mechanisms of seed germination under low-temperature in rapeseed, we crossed a tolerant cultivar with a susceptible genotype to develop a mapping population of 574 F_2 progenies. Two quantitative trait loci (QTLs) for low-temperature germination (LTG) were detected on chromosome A09 (named *qLTGA9-1*) and C01 (named *qLTGC1-1*) using QTL-seq method, and confirmed via linkage analysis in the mapping population. *qLTGA9-1* was mapped to a 341.86-kb interval between SSR markers *Nys9A212* and *Nys9A215*. In this region, 69 genes including six specific genes with moderate or high effect function variant were identified based on Ningyou7 genome sequence. *qLTGC1-1* was mapped a 1.31-Mb interval between SSR markers *Nys1C96* and *Nys1C117*. In this region, 133 genes including five specific genes with moderate effect function variant were identified based on Ningyou7 genome sequence. These specific genes within the two QTLs could be targets in rapeseed breeding programs and further studies for cold tolerance.

Introduction

Rapeseed (*Brassica napus* L.) is a major winter crop in the Yangtze River Basin, China, with sown area of 6.6 million hectares and annual production of 13.5 million tons[1,2]. Along with technology advancement and labour costs rise, the cultivate patterns of rapeseed is gradually changed into no-tillage direct seeding[3]. Recent years, the late direct seeding area for rapeseed is continuously increased as the intensive cropping development. However, since the temperature at late sowing time in China are frequently below 10°C, the direct sowing of rapeseed growth are easily affected due to low germination rates and seedling under low-temperature conditions[4]. Low-temperature limits seed germination and seedling growth, which ultimately affect yields[4,5]. The germination stage of rapeseed is sensitive to temperatures below 10°C[6,7]. Low-temperature during germination stage is an important problem for rapeseed cultivation. Selecting superior varieties of rapeseed with a high rate of low-temperature germination (LTG) has become an important breeding goal under late direct seeding cultivation.

Different cultivars respond differently to low-temperature, and the differences are largely controlled by genetic factors. The LTG ability of crops is a complex trait that controlled by quantitative trait loci (QTLs)[4,5,8]. Lots of QTL mapping studies for LTG have been conducted in crops, such as rice, maize, soybean and wheat[9,10,11,12,13,14,15]. In rapeseed, there was a wide genotypic variation of LTG for many studies[16,17], and a few QTLs associated with low-temperature stress during seed germination and seedling stages were reported. Xian et al. (2017)[4] identified several different expressed genes associated with low-temperature tolerance in rapeseed through transcriptome analysis. Luo et al. (2021)[7] detected 22 QTLs associated with low-temperature tolerance during seed germination and seedling stages through genome-wide association study (GWAS). Except that, to our knowledge, the genetic study of rapeseed germination under low-temperature is still rare. To date, very few markers associated with LTG have been developed for

rapeseed breeding. Thus, it is critical to find and identify new LTG QTLs for further fine-mapping or molecular breeding to accelerate breed low-temperature tolerant varieties of rapeseed during seed germination stage.

QTL associated with interest traits can be identified through different QTL mapping approaches including QTL-seq, which is an effective approach via bulked segregant analysis (BSA) using next-generation sequencing (NGS) for mapping QTL[18]. QTL-seq has been successfully used to rapidly identify QTLs for different traits in some field crops, such as spikelet fertility under heat stress in rice[19], plant height in wild soybean[20], cold tolerance in wild rice[21], etc. In this study, we employed the $F_{2:3}$ populations, derived from two inbred lines Huyou21 (tolerant to low-temperature stress) and 3429 (susceptible to low-temperature stress), to detect the QTL related to rapeseed LTG by QTL-seq method. The goals of this study were to: (i) analyze seed germination of this population under low-temperature; (ii) identify QTL for LGT from this population by QTL-seq method; (iii) develop simple sequence repeat (SSR) markers of the LTG QTLs for further fine-mapping or molecular breeding.

Results

Comparison of LTG in the parental lines

Based on a preliminary screening experiment for LTG, the tolerant genotype (Huyou21) and susceptible genotype (3429) were selected for further study. At optimal temperature (20°C), the two parents had similar germination rate (higher than 95%) within 4 days after imbibition (DAI). At low-temperature (8°C), Huyou21 exhibited excellent tolerance to cold stress, with a mean germination rate higher than 90% within 4 DAI. In contrast, 3429 showed high susceptibility at 8°C, with a mean germination rate lower than 40% within 4 DAI (Fig. 1).

Phenotypic analysis in $F_{2:3}$ families and construction of bulks

Based on the result, Huyou21 and 3429 were chosen to construct a segregating population for a genetic study of LTG. The $F_{2:3}$ families showed pronounced variation and segregation of cold stress tolerance or sensitive (Fig. 2). The LTG tolerance distribution was continuous and approximately normal in this $F_{2:3}$ families, indicating a quantitative inheritance of LTG. The phenotypic trait indices (PTI) of F_2 progeny ranged from 0.19 to 2.58. When PTI was more than 2.33, the F_2 plant was identified as extremely tolerance to low-temperature under 8°C (referred to LT). When PTI was lower than 0.99, the F_2 plant was identified as extremely sensitive to low-temperature under 8°C (referred to LS). The LT and LS individuals were selected to establish LT- and LS-bulks, respectively.

Resequencing and mapping of reads

The two bulks (LT-bulk and LS-bulk) with extreme phenotypes (the tolerant and sensitive pools) together with two parents were subjected to prepare the libraries for Illumina sequencing. A total of 319,059,584

reads for LT-bulk, 335,343,844 reads for LS-bulk, 323,983,156 reads for tolerant parent (Huyou21) and 337,990,498 reads for tolerant parent (3429) were generated. Alignment of the reads generated from the bulks to the Ningyou7 rapeseed genome sequence[22], achieved 36.52× and 37.58× read depth and 99.17% and 99.13% coverage for LT-bulk and LS-bulk, respectively. Similarly, for the parents, the alignment of 36.41× and 38.26× read depth and 95.73% and 97.31% coverage were obtained in Huyou21 and 3429, respectively (Table 1). In total, 5,600,575 genome-wide SNPs and 1,215,792 InDels for LT-bulk, and 5,604,534 SNPs and 1,217,972 InDels for ST-bulk were identified by comparison with the reference genome (Table S1 and Fig.S1).

Table 1
Summary of the sequencing results for the parental lines and two bulks.

Genotypes	Clean reads No.	Clean base (Gb)	Read alignment (%)	Average depth (×)
LT-bulk	319,059,584	47.48	99.17%	36.52
LS-bulk	335,313,844	49.89	99.13%	37.58
Huyou21	323,983,156	48.17	95.73%	36.41
3429	337,990,498	50.36	97.31%	38.26

Candidate genomic regions for LTG by QTL-seq

To identify candidate genomic regions related to LTG, the SNP index was calculated for each bulk. Then Δ(SNP-index) was calculated and plotted against the genome positions by combining the information of the SNP-index in LT- and LS-bulks (Fig. 3). Δ(SNP-index) was calculated by the SNP-index of LT-bulk minus the SNP-index of LS-bulk, and plotted across the 19 rapeseed chromosomes to mapped the putative genomic regions associated with the phenotype for LTG. At the 95% statistical level, two QTL regions on two chromosomes A09 and C01 were detected based on Δ(SNP-index) plot (Fig. 3C). The peak of *qLTGA9-1* was located between 40.00-Mb and 49.73-Mb on chromosome A09 (Fig. 3D) and *qLTGC1-1* was located between 38.90-Mb and 55.38-Mb on chromosome C01 (Fig. 3E). The results revealed that there were one QTL related to LTG at the 9.73-Mb region of chromosome A09, named *qLTGA9-1*, and another QTL at the 16.48-Mb region of chromosome C01, named *qLTGC1-1*.

Marker development and QTL fine mapping

In order to examine the result of QTL-seq, total of 351 SSR markers including 163 primers in the 9.73-Mb candidate region (*qLTGA9-1*) on chromosome A09 and 188 primers in the 16.48-Mb candidate region (*qLTGC1-1*) on C01 were developed and genotyped in Huyou21 and 3429. Among which 17 primer pairs (eight on A09 and nine on C01) produced steady and clear polymorphic bands between two parents (Table 2). These polymorphic markers were selected for linkage map construction, and performed for linkage analysis in the 574 F_2 individuals with the phenotype of LTG. Combining the results of the genetic map and the phenotypic analysis for LTG, both QTL were found on the predicted regions (Fig. 4). The QTL

qLTGA9-1 was mapped in a 1.78-cM interval between the tightly linked markers, *Nys9A212* and *Nys9A215*, which only explained 5.93% of the phenotypic variation for LTG but had a higher LOD value of 8.43 (Fig. 4 and Table 3). Meanwhile, *qLTGC1-1* was mapped in a 16.40-cM interval between the tightly linked markers, *Nys1C96* and *Nys1C117*, its phenotypic variation was 5.39% and LOD value was 6.57 (Fig. 4 and Table 3).

Table 2
Primer sequences of polymorphic markers at the candidate regions for LTG.

Markers	Primer sequences		Chr[a]	Location[b]
	Forward (5'-3')	Reverse (5'-3')		
Nys9A204	GCATGTATAACTCCTTGAATCC	CCACAAGAAATTAGAGGTCG	A09	43716935–43717137
Nys9A207	TACTCCTTTGGAAGGAAACA	TCCCTCTCCATCTGAAAATA	A09	44059061–44059257
Nys9A208	ATTCTGTGTATCCCATTTCG	GAGGAGGTTTCTGAGGAGTT	A09	44230061–44230241
Nys9A145	TTCAGTAAAGCTGACACACG	CGAGAGGTTATTAGGGGTTT	A09	44611834–44612080
Nys9A212	CAAAAGAGGGAATTTCAGTG	TGTCTCTAGTGAGAAAGCATTG	A09	44721694–44721885
Nys9A215	AACACACAGACATCGAGACA	TGAGATTGAAAGAGAAGGGA	A09	45063742–45063891
Nys9A216	CCCTGGATATAGCTTGTGAT	AGACCTTTTTCATTGTCAGG	A09	45165658–45165884
Nys9A218	TCTGGACAGCATCTTTAGGT	CTCAGACAACTCAGCAATCA	A09	45389536–45389768
Nys1C90	ATAAATAGCCCCACGTCTCT	ACAGAGGAAATCGAAACAGA	C01	47748257–47748503
Nys1C91	GGGAGGTTCTAGGACAAAAT	AGTGACAGATGGGATCAAAC	C01	47788771–47789026
Nys1C96	GAGCTTAAGGGCTCTTCTTC	TAGGAGACTTGACCATCCAC	C01	48189600–48189816
Nys1C46	CTGAAACCCAAAAATAGCG	TAGATCCAGCTAAGTACCCG	C01	48690912–48691125
Nys1C191	CACGTACAATCAGTCAACCA	CCATCACCATGAAAATCTCT	C01	49158881–49159050
Nys1C117	CTGGATAAAAAGAAACGTGG	TCTCTTCTTCATGCCTCTGT	C01	49503123–49503320
Nys1C137	CTTTCTCCTCTTTTCCGATT	CCTTGCTTAGACTTCTTGGA	C01	51668433–51668607
Nys1C142	TCCTTACCGCAAGAAACTAC	CGACGAGGAGTATGAGTAGG	C01	52183392–52183583
Nys1C76	TTCTCTCTTGGGAAAAGTCA	AGCCTTTGAAGACTAAACCC	C01	54524191–54524377

[a]Chr is the chromosome. [b]Location is the marker position on the reference genome sequence.

Table 3
Statistics of LTG QTL identified at 4 DAI in $F_{2:3}$ families.

QTL	Chr[a]	Marker Interval	Distance (cM)[b]	LOD	PVE (%)[c]	Add[d]	Dom[e]
qLTGA9-1	A09	Nys9A212-Nys9A215	1.78	8.43	5.93	0.13	0.14
qLTGC1-1	C01	Nys1C96-Nys1C117	16.40	6.57	5.39	0.08	0.18

[a]Chr is the chromosome of rapeseed. [b]Distance means the distance between the left and right marker of its mapping QTL. [c]PVE means the phenotypic variation of LTG in $F_{2:3}$ families by each QTL. [d]Add is the additive effect. [e]Dom is the dominant effect.

Based on the physical position of the tightly linked markers, *qLTGA9-1* was physically located in 341.86-kb interval between 44.72-Mb and 45.06-Mb on rapeseed chromosome A09, and *qLTGC1-1* was physically located in 1.31-Mb interval between 48.19-Mb and 49.50-Mb on chromosome C01. According to the annotation information of Ningyou7 genome sequence[22], 69 and 133 predicted genes were contained in *qLTGA9-1* and *qLTGC1-1* region, respectively. Based on the sequencing results for each QTL, 74 SNP and 25 InDel mutations related to 32 predicted genes in *qLTGA9-1* region, and 162 SNP and 26 InDel mutations related to 35 predicted genes in *qLTGC1-1* region were screened (Table S2 and Table 4). Of these predicted genes, only one gene in *qLTGA9-1* region with high effect variant (frameshift insertion/deletion) and five genes in *qLTGA9-1* and *qLTGC1-1* region with moderate effect variant (missense mutation), respectively (Table 4 and Table 5). These genes with moderate or high effect variants may be related to low-temperature tolerance at generation stage.

Table 4
Candidate genes contained in the intervals of *qLTGA9-1* and *qLTGC1-1*.

QTL	QTL region (Mb)[a]	Number genes	Number genes with high effect variant[b]	Number genes with moderate effect variant[b]
qLTGA9-1	44.72–45.06	32	1	5
qLTGC1-1	48.19–49.50	35	0	5

[a]QTL region defined as union of QTL-seq and linkage mapping credible intervals. [b]Functional effects of variants (moderate or high) determined by snpEff tool.

Table 5
Annotation of the candidate genes with moderate (high) effect variants in *qLTGA9-1* and *qLTGC1-1* regions.

QTL	Gene ID	Start base - end base (bp)[a]	Functional effect[b]	Chain	Predicted function[c]
qLTGA9-1	*ChrA09g005501*	44842926–44843909	Moderate	Forward	Formiminotransferase
	ChrA09g005502	44845212–44845795	Moderate	Reverse	–
	ChrA09g005507	44864501–44865259	Moderate	Forward	Protein phosphatase 2C
	ChrA09g005509	44869664 – 44570813	High	Forward	Aminotransferase class I and II
	ChrA09g005523	44950207–44952401	Moderate	Forward	DRG Family regulatory protein
	ChrA09g005524	44954947–44959392	Moderate	Forward	DRG Family regulatory protein
qLTGC1-1	*ChrC01g004357*	48588162–48594007	Moderate	Forward	SWEET sugar transporter
	ChrC01g004359	48596431–48597483	Moderate	Forward	PHD-finger
	ChrC01g004400	49078002–49082772	Moderate	Forward	Plant invertase/pectin methylesterase inhibitor
	ChrC01g004405	49166002–49166734	Moderate	Reverse	–
	ChrC01g004406	49193433–49194020	Moderate	Forward	–

[a]Physical position of predicted gene was from the reference genome of Ningyou7. [b]Functional effects of variants (moderate or high) determined by snpEff tool. [c]Predicted function of candidate gene was based on Ningyou7 annotation information. – indicates that no putative conserved domains have been detected.

Based on Ningyou7 annotation information, seven of these predicted genes (four genes in *qLTGA9-1* and three genes in *qLTGC1-1*) encode proteins associated with plant growth or response to temperature stresses: *ChrA09g005501* encoding a formiminotransferase, *ChrA09g005507* encoding a protein phosphatase 2C (PP2C), *ChrA09g005509* encoding an aminotransferase, *ChrA09g005523* and *ChrA09g005524* encoding DRG family regulatory proteins (DFRP), *ChrC01g004357* encoding a SWEET sugar transporter, *ChrC01g004359* encoding a PHD-finger and *ChrC01g004400* encoding a plant invertase/pectin methylesterase inhibitor (PMEI) (Table 5).

Discussion

Recent years, the late direct seeding area for rapeseed in China is continuously increased as the intensive cropping development. Whereas the rapeseed germination is easily affected by low-temperature with the delay of seeding times[4]. Low-temperature sensitively at the germination stage is a major challenge for rapeseed cultivation under the late direct-seeding conditions in China[4,7]. It is a breeding goal to select varieties with well germination under both optimal and stressed conditions to adapt the changing temperature under late direct seeding cultivation. In our previous studies, the seed germination rate showed a high degree of variability among the rapeseed genotypes under low-temperature conditions. The genotypic variability could benefit the LTG studies of rapeseed breeding to deal with cold stress under late direct-seeding condition, and also other cold conditions during sowing.

Previous studies revealed that LTG varied greatly among plant species, which significantly affected by inheritance and environmental factors and controls by QTLs and multi-genes[4,5,8]. Some QTL mapping studies for LTG have been conducted in many crops but few QTL or genes for LTG were reported in rapeseed. QTL-seq is an effective approach for QTL identification by combined the BSA and NGS[18]. The method has been successfully used to rapidly identify QTLs of different traits in rapeseed[23,24]. In this research, we employed a segregating population to detect the LTG QTL of rapeseed using QTL-sEq. After investigating the LTG in the generations from the population derived from the 3429 × Huyou21 cross, we found the LTG of these populations are governed by incomplete dominant nuclear genes or QTL (Table S3). QTL-seq revealed that there were two QTLs (namely *qLTGA9-1* and *qLTGC1-1*) associated with LTG on chromosomes A09 and C01, and both of them were verified with classical QTL analysis through map construction. *qLTGA9-1* position was mapped between the flanking SSR markers *Nys9A212* and *Nys9A215* and *qLTGC1-1* was mapped between *Nys1C96* and *Nys1C117*. In our study, the position of the QTL (*qLTGA9-1*) on chromosome A09 was mapped around 30.2-Mb apart from each other based on the physical position of linkage markers on ZS11 genome sequence[25]. GWAS mapping from Luo et al. (2021)[7] showed the candidate genes to be around the 3.0-Mb position on chromosome A09 based on ZS11 genome sequence[25] related to seed vigor under low-temperature. As the physical distance between the locus and the QTL we identified was more than 27 Mb probably rules out, we believe that the QTLs including *qLTGC1-1* we found were novel in rapeseed for LTG.

To recapitulate the physical position based on the Ningyou7 genome sequence[22], 69 and 133 genes were predicted in *qLTGA9-1* and *qLTGC1-1*, respectively, and 11 of these predicted genes with moderate/high effect variant according QTL-sEq. Among these predicted genes, *ChrA09g005507* in *qLTGA9-1* region encoding a PP2C and *ChrC01g004357* in *qLTGC1-1* region encoding a SWEET protein. PP2C is a key player in ABA signal transduction, which acts an important role in crop seed germinations under cold stress[26,27,28], while SWEET protein is an important type of plant sugar transporter family and plays a crucial role in seed germination and stress response[29,30]. In addition, *ChrA09g005523* and *ChrA09g005524* in *qLTGA9-1* region encoding DFRPs, *ChrC01g004359* and *ChrC01g004400* in *qLTGC1-1* region encoding a PHD-finger and a PMEI, respectively. Similarly to PP2C or SWEET, the DFRP, PHD-finger and PMEI protein

also involved in defense responsive or related against abiotic stress[31,32,33]. Future studies will be intended to validate these candidate genes including gene sequence analysis and its functional validation.

Materials And Methods

Plant materials and phenotypic evaluation

The parents used for developing the mapping population were Huyou21 (tolerant) and 3429 (susceptible) (Fig. 1). Seeds of cultivars Huyou21 and 3429 were obtained from Shanghai Academy of Agricultural Sciences. Huyou21, was developed from a double cross between 9714/9711 and 84004/8920, is widely cultivated in the lower reaches of the Yangtze River Basin, China. 3429, used as the female, is a new line derived from self-cross plant of the hybrid variety Qinyou99. The 3429 × Huyou21 hybrids were advanced from the F_1 generation by selfing to yield an $F_{2:3}$ families for mapping of LTG.

In this study, the F_2-derived F_3 population seeds from 3429 × Huyou21 were evaluated for LTG. Five hundred healthy and plump F_3 seeds per F_2 plant were placed on two layers of filter paper with 15 mL distilled water in Petri dishes (9 cm inner diameter). The germination experiment was conducted in a low-temperature incubator set at 8°C and arranged in a completely randomized design with three replications. The number of germinated seeds (N_1) was counted at the 4th day after imbibition (DAI). Then remove the seeds that not germinated to normal-temperature (20°C) to start the recovery process for 3 days, and counted its germinated seeds number (N_2) to exclude the low-vigor seed influences. Germination was defined by the radicle emergent 2–3 mm from the seed. The seed germination under low-temperature was calculated as relative germination rate (RGR, %) = N_1 / (N_1 + N_2) × 100%. The PTI of $F_{2:3}$ families for QTL mapping of LTG were calculated according to the formula: PTI = 2 × arcsin(X) / [arcsin(P_1) + arcsin(P_2)], where in X is the grand mean for each RGR of $F_{2:3}$ family. P_1 and P_2 is the grand mean for each RGR of Huyou21 and 3429, respectively.

Sample bulking and DNA isolation

Total of 574 F_2 individuals from the cross 3429 × Huyou21 was selected to build DNA bulks for QTL-sEq. Genomic DNA from young leaves of F_2 individuals were isolated using Plant Genomic DNA Kit (TIANGEN, China) followed the instructions for fine mapping. Thirty individuals for low-temperature tolerant (LT) bulk and another 30 individuals for the low-temperature sensitive (LS) bulk were selected from the F_2 population based on the extreme phenotype for PTI under the low-temperature (8°C) of $F_{2:3}$ families, respectively. Genomic DNA from LT-bulk, LS-bulk and their parents (Huyou21 and 3429) were isolated using Plant Genomic DNA Kit (TIANGEN, China) followed the instructions. DNA quality and concentration was examined by electrophoresis on 1% (w/v) agarose gels.

Illumina sequencing and analysis of NGS data

Test qualified genomic DNA samples for LT-bulk, LS-bulk and two parents were sent to Shanghai OE Biotech Co., Ltd (China) to construct libraries with insert size of 350–500 bp using the TruSeq DNA LT

Sample Prep kit and sequenced at 150-bp pair-end reads using an Illumina Xten platform. Raw data generated from Illumina sequencing was subjected to a quality control using Trimmomatic Version 0.36[34]. The filtered clean reads from both parents and two DNA bulks were aligned to the rapeseed genome sequence[22] using BWA software and single nucleotide polymorphism (SNP) calling was performed with SAM tools[35]. The SNPs and InDels were detected and classified based on the positions in the reference genome. The average of SNP-index for each pool was calculated in a 1 Mb sliding windows with a 10 kb increment. The △(SNP-index) was calculated by subtracting the SNP-index of LS-bulk from that of LT-bulk. All SNP-index and △(SNP-index) were calculated as previously described[18,36] for all positions to identify LTG-related QTLs.

SSR markers analysis and QTL fine mapping

LTG-related QTLs identified by QTL-seq were validated and fine mapped through traditional QTL mapping method. A total of 351 SSR markers in the predicted regions were mined from the whole-genome sequence[22]. The SSR markers were used to survey the polymorphism between parents, which were designed with SSR Locator[37] based on the parameters as previously described[38]. The newly developed markers were named *NysX(A/C)Y* markers, where *Nys* represents the microsatellite from the physical sequence of Ningyou7 rapeseed, the number "*X*" indicates the chromosome in subgenome (A or C) and "*Y*" represents a numerical code for newly designed marker. Polymerase chain reaction (PCR) amplification and the PCR products detection was conducted as previously described[39] with minor modifications. Polymorphic markers were further selected to analyzed F_2 population to construct a linkage map for QTL fine mapping. The genetic linkage map was drawn using the MAP functionality and QTL was conducted using BIP functionality in QTL IciMapping v4.1[40] (Lei et al., 2015). A map distance (cM) was calculated using the Kosambi mapping function[41], and ICIM-ADD was selected as mapping methods[42]. The LOD thresholds and recombination frequency value was set at 3.0 and 0.30, respectively. Identified QTL were designated by the chromosome number followed by *qLTG*.

Permission Statement

All the experiments on plants, including the collection of rapeseed materials, were performed in accordance with relevant guidelines and regulations.

Conclusions

A total of 574 $F_{2:3}$ families were constructed to understand the genetic mechanisms of seed germination under low-temperature in rapeseed. Based on the QTL-seq and linkage analysis of the populations, two QTLs were detected from 'Huyou21'. One QTL was mapped to a 341.86-kb interval between SSR markers *Nys9A212* and *Nys9A215* on rapeseed chromosome A09, and another was mapped to 1.31-Mb interval between *Nys1C96* and *Nys1C117* on chromosome C01. These results supplied important basis for further studies and candidate genes cloning of cold tolerance in rapeseed.

Declarations

Data availability

This whole genome resequencing reads used in QTL-seq has been deposited in the National Center of Biotechnology Information Sequence Read Archive (SRA) under BioProject accession number PRJNA751740.

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Author contributions statement

JZ designed and performed the experiments, collected and conducted the data analysis, and wrote the manuscript. XZ provided the genetic materials and assisted in phenotype investigation. WW, MJ and LY worked on the materials sowing. JZ and XZ conceived the study and finalized the manuscript. All authors read and approved the final version of paper.

Ethics declarations

Competing interests

The authors declare no competing interests.

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Figures

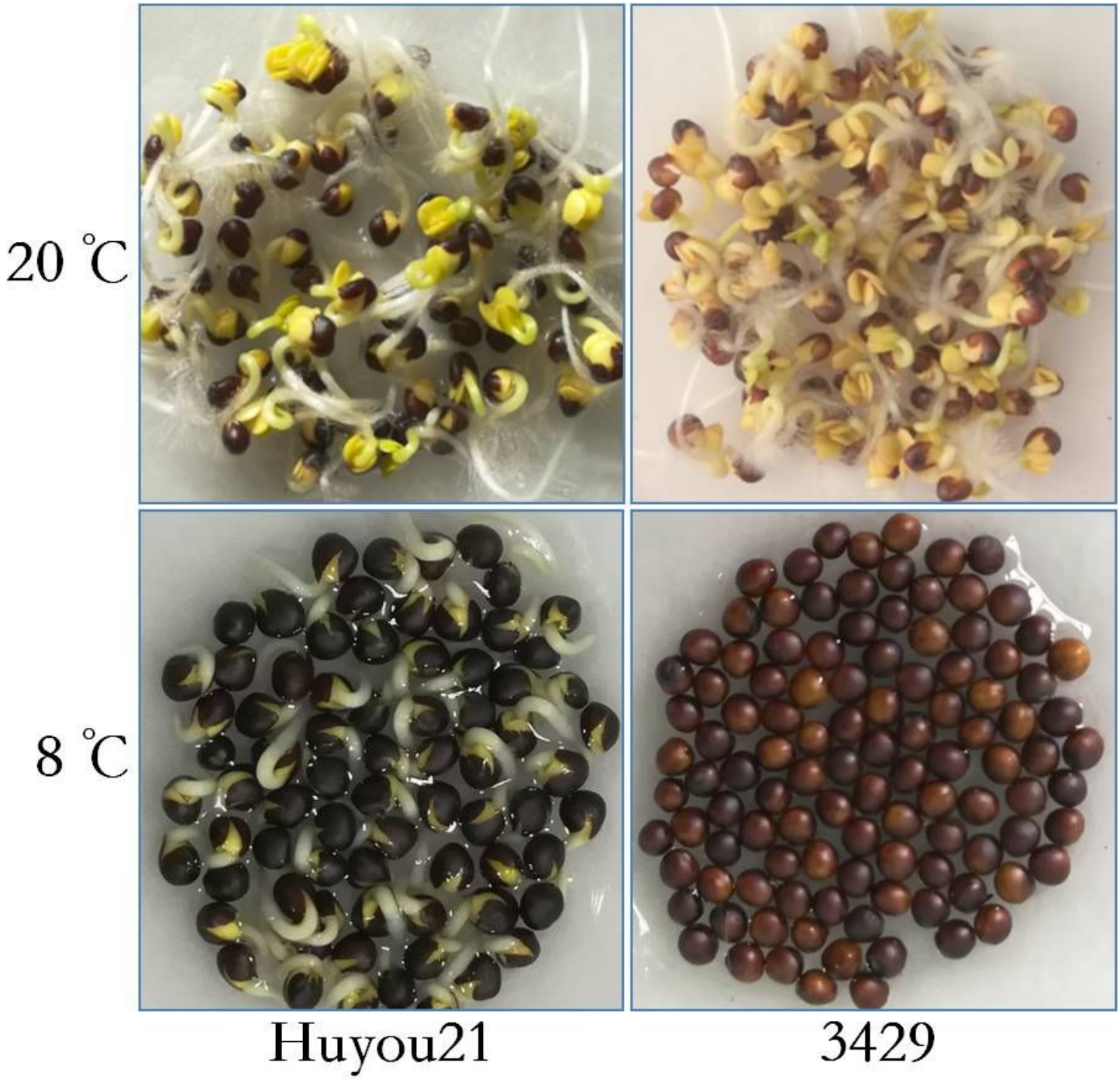

Figure 1

Phenotype of Huyou21 and 3429 for 4 DAI under 20°C and 8°C, respectively.

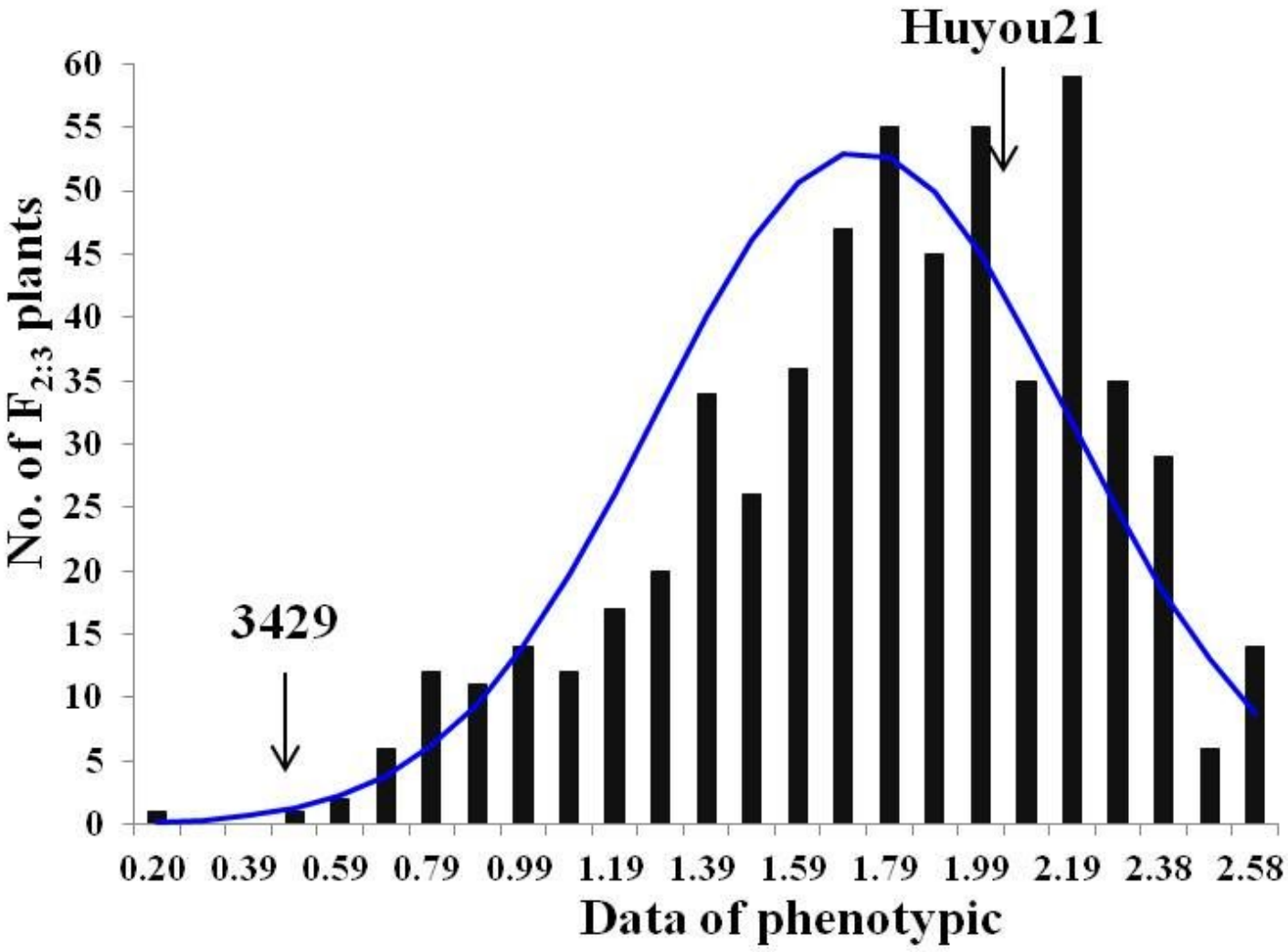

Figure 2

Distribution of the phenotypic in F2:3 population for 4 DAI at 8°C.

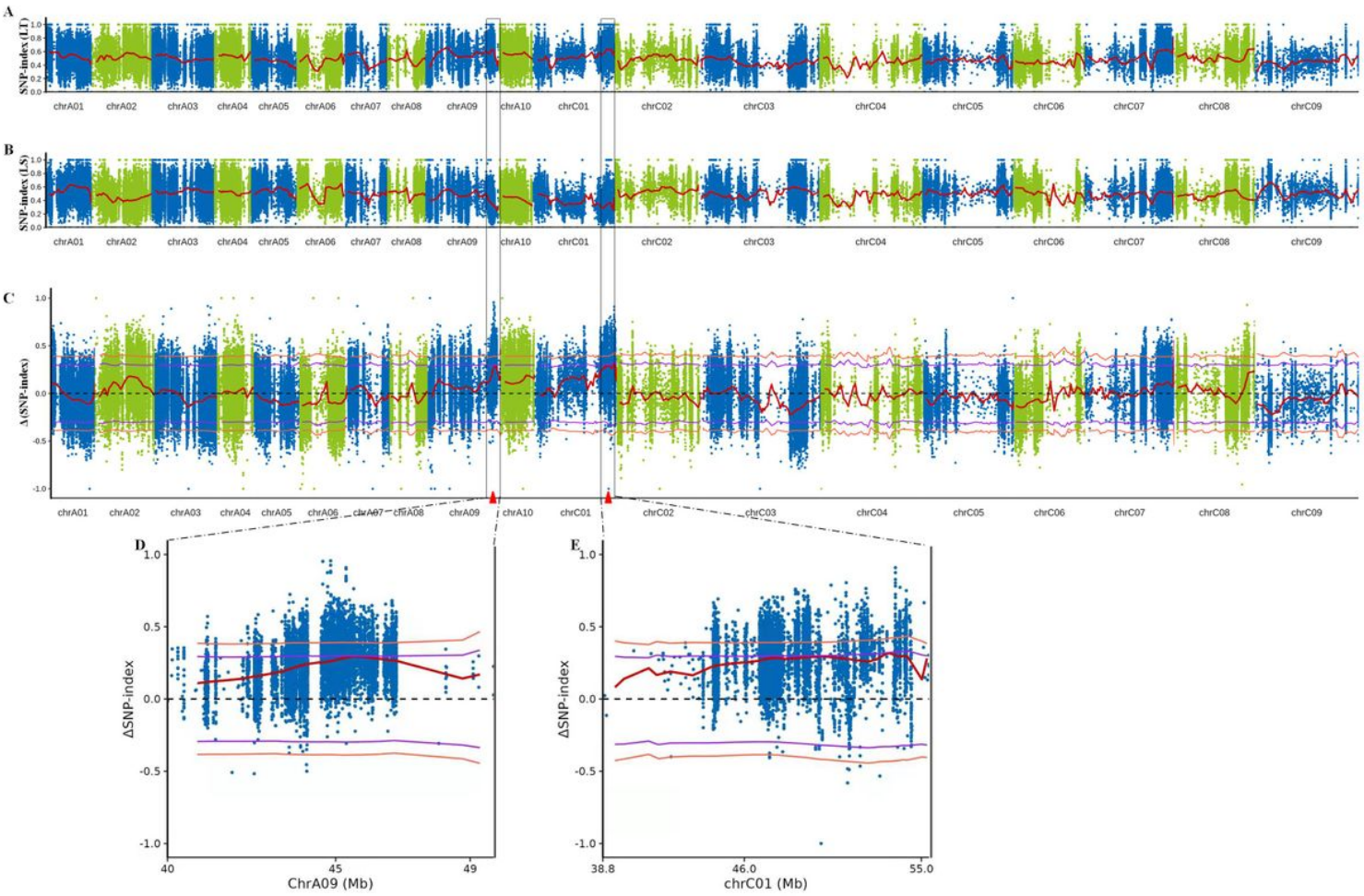

Figure 3

Identification of LTG QTL in an F2 populations by QTL-seq. X-axis represents the chromosomes of rapeseed and Y-axis represents the SNP-index. Δ(SNP-index) plot with statistical confidence interval under the null hypothesis of no QTL (purple, P < 0.05; orange, P < 0.01). A, B and C represent the SNP-index plots of LT-bulk and LS-bulk, and the Δ(SNP-index) plot of 19 rapeseed chromosomes from QTL-seq analysis, respectively. D and E represent the significant genomic regions of 40.00-49.73 Mb on chromosome A09 and 38.90-55.38 Mb on chromosome C01 identified for LTG, respectively.

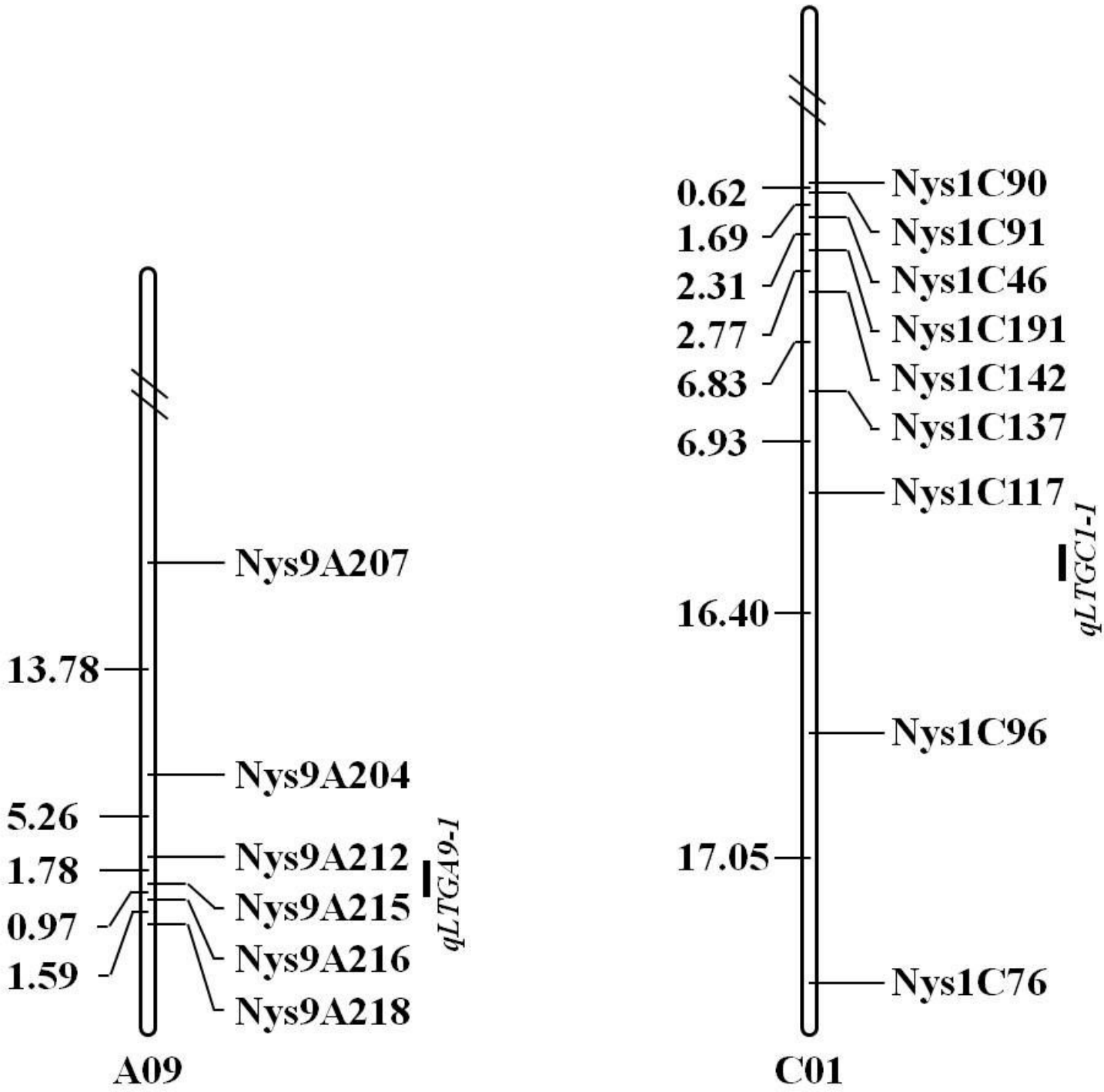

Figure 4

Fine mapping of the LTG QTLs in an F2:3 families derived from 3429 × Huyou21. The number at left are map interval sizes in Kosambi centiMorgan (cM) units and at right are the SSR markers on chromosome A09 and C01, respectively.

Supplementary Files

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- Fig.S1.jpg
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- TableS2.xlsx
- TableS3.xlsx