Identification of QTL for Cold Tolerance at the Booting and Flowering Stage in Rice (Oryza sativa L.)

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Abstract

Rice growth and productivity are greatly affected by cold stress, which is likely to become more threatening to high and stable yield of rice. To identify cold tolerance at the booting and flowering stage in rice, a set of recombinant inbred lines (RIL) was developed by crossing a cold-tolerant *japonica* cultivated variety, Jileng1 (JL1), with a cold-sensitive *indica* cultivated variety, Milyang23 (MY23). The seed setting rate (SST) of the parents and RIL populations were investigated under different temperature environments, then the SST and cold stress tolerance index (CSTI) under the natural low-temperature were used to evaluation the cold tolerance and quantitative trait locus (QTL) mapping. Nine QTL were detected on chromosome 1, 2, 3, 5, 7, 11 and 12, with log-likelihood (LOD) value ranging from 2.64–4.76, these QTL explain the phenotypic variance explained (PVE) range from 3.34 to 12.02%. Among of theses QTL, three QTL *qSST1*, *qSST5* and *qSST12* were detected on different years, and they were considered as stable expression QTL. *qSST5 was* identified on chromosome 5 marker between CMB0526.3 and ID5014265, which many QTL related to cold resistance have been identified in previous studies, so that this QTL was considered as a major QTL for cold tolerance. In addition, thirteen QTL with environmental interactions were detected, and then the additive QTL were all involved in environmental interactions. These result showed that environmental interactions have an significant effective on cold tolerant in rice. The stable expression major QTL identified will help to fine mapping these cold tolerance QTL and provide the gene resources to cultivation cold tolerant variety in rice.

Introduction

Rice (*Oryza sativa* L.) is the main staple food crop worldwide, feeding more than half the world's populations (Sasaki and Burr 2000). Rice is a cold-sensitive crop, low-temperature stress have a negative influences on the vegetative and reproductive stages, the booting and flowering stage are the most sensitive period, which have a fatal effect on production in the worldwide (Xu et al. 2008; Cruz et al. 2013). At the booting and heading stage, cold stress affects panicle growth, including the pollen activity, seed fertility and seed size, which ultimately results in decreased yield (Li et al. 2017). Therefore, improving cold tolerance is one of the most important goals and research hotspot for breeders in rice, it would be beneficial to cultivate cold-resistant rice varieties.

Cold tolerance is a complex trait by quantitative manner and have a complicated genetic basis, controlled by a large number QTL and affected simultaneously by environment (Andaya and Mackill 2003a; Zeng et al. 2009; Zhou et al. 2010). Many QTL related to cold tolerance at the booting stage have been reported, these QTL have been identified on chromosomes 1, 2, 3, 5, 6, 7, 9 and 12 (Andaya and Mackill 2003b). *Ctb1* and *Ctb2* was related to the cold resistance, which located on chromosome 4 (Saito et al. 2001, 2004). *Ctb1* has been fine mapped in a 17-kb region that contains two candidate genes, encode a F-box protein and a ser/thr protein kinase, the former have a significant correlation with cold tolerance (Saito et al. 2010). *qCTB8* for cold tolerance was detected on the short arm of chromosome 8 (Kuroki et al. 2007). Xu et al.(2008) has been evaluated cold tolerance by spikelet fertility of the main panicles from the parents and BC_5F_3 populatin, and then eight QTL was identified on chromosomes 1, 4, 5, 10 and 11.

Subsequent research *CTB4a* and *qCTB10-2* have been fine mapped, the *CTB4a* encodes a conserved leucine-rich repeat receptor-like kinase (Zhang et al. 2017), the *qCTB10-2* has been delimited to a 132.5-kb region containing 17 candidate genes, four genes was related to cold treatment inducible (Li et al. 2017). A major QTL *qPSST6* related to cold tolerance was located on chromosome 6 (Sun et al. 2018). Tang et al. (2019) identify cold tolerance QTL at the reproductive stage in rice by two RIL populations, 17 QTL was detected on chromosomes 1, 3–6, 8, 11 and 12.

In the recent years, numerous QTL for cold-resistant have been mapped at the booting stage, and then several QTL have been stable detected in different environments, however, there only a few QTL have been cloned and applied in rice breeding. Previous researchers, to detect stable QTL regulate to the cold tolerance, the F_2 and $F_{2:3}$ populations was developed by crossing JL1 (cold-tolerant *japonica* cultivated variety) with MY23 (a cold-sensitive *indica* cultivated variety), which some major QTL have been found and have a positive effective in increase cold tolerance in the seedling stage and booting stage, respectively (Han et al. 2005a, b). In this study, a set of RIL populations developed by crossing from these parents was used the experimental material, the SST and CSTI as evaluate the cold tolerance at the booting and flowering stage in rice, we will detected these stable expression QTL for natural low-temperature tolerance. The research results will provide a basis for these major QTL fine mapping and successfully cultivate the strong cold-resistant varieties in rice.

Materials And Methods

Experimental materials

A set of RIL populations, containing 253 lines, was developed by crossing between the cold-tolerant *japonica* cultivated variety JL1 as the donor and the cold-sensitive *indica* cultivated variety MY23 as the receptor using the single seed descent (SSD) method. The F_2 generation from JL1 × MY23 was subjected to more than ten rounds of self-pollination to generate the RIL populations.

Field experiment

The natural low-temperature test was carried out in the field of Yunnan Academy of Agricultural Sciences, Songming (SM), Yunnan province, China (25°05'N, 102°72'E, 2136 m). Between early June and late September, the daily minimum temperature is approximately 15–19°C (Fig. 1), which is a suitable temperature to test the cold tolerance at the booting and flowering stage in rice. The RIL populations and their parents were sown in Songming in 2016 (E1), 2017 (E2) and 2018 (E3). Moreover, all lines were sown in the Changping Test Field of the Institute of Crop Science, Chinese Academy of Agricultural Sciences (Beijing, BJ) in 2018 (Eb1) and 2019 (Eb2).

The heading date was investigated for three years under natural low-temperature conditions. The parents and RIL populations were sown in early April in 2016, and the heading and flowering periods were mainly concentrated in July 5 to August 27. All lines were planted in late April in 2017 and 2018, and the heading and flowering periods occurred from July 15 to September 29 (Fig. 1). Therefore, the varieties experienced

the critical temperature considered cold stress at the booting and flowering stage. We estimated the SST of parents and RIL populations when plants reached the maturity stage. The SST phenotype of the RIL lines and parents were evaluated using the mean values of five main panicles in each line, and the phenotypic value of each line was the mean of two replicates. The average SST of three years in Songming (aSSM) and the CSTI were also used for QTL mapping. The CSTI was defined as the ratio of the aSSM to that the average SST in Beijing (aSBJ).

DNA extraction and genotyping

Genomic DNA was extracted using the CTAB method. A total of 291 polymorphic markers, including 114 single nucleotide polymorphism (SNP), 62 sequence-tagged sites (STS) and 115 Simple Sequence Repeats (SSR) markers, evenly distributed throughout the entire genome of rice were used to genotype the RIL populations. The genotyping of SNP markers were used by SNP chips, the same genotype as JL1 was “2”, and the same genotype as MY23 was “0”. The PCR products of SSR and STS were separated using 8% polyacrylamide gel electrophoresis. At the same migration rate position, the amplified polymorphic DNA segments, that were the same as those of JL1 were recorded as “2”, that were the same as those of MY23 were recorded as “0”. In addition, missing segments were recorded as “-1”.

Linkage map construction and QTL analysis

The genetic linkage map was constructed using JoinMap4 software (Ooijen 2006) with 114 SNP, 62 STS and 115 SSR markers, covering 12 chromosomes. The genetic linkage map was drawn with Mapchart software (Voorrips 2002). Inclusive composite interval mapping (ICIM) method was used to determined the QTL by QTL IciMapping 4.2 (http://www.isbreeding.net/) with the minimal logarithm of the LOD score of 2.50. The QTL naming convention was as described by McCouch et al. (1997).

Results

Phenotypic variation of cold tolerance in the RIL populations

The SST was assessed for the parents and RIL populations in Beijing and Songming. The climatie of Songming had natural low-temperature conditions at three years. Due to the noncompatibility of the *indica* and *japonica* crossings, varieties with SST less than 70% were eliminated in Beijing at 2018 and 2019. Finally, 219 lines were used for this research. The phenotypic evaluations and comparisons of SST for JL1, MY23 and the RIL popilations in Beijing and in Songming (Fig. 2 and Table 1). JL1 showed a higher SST than MY23 under different environments. The average of SST of JL1 was 93.39%, higher than that of MY23, 78.90% in Beijing environmental conditions, but there was no significantly different. In Songming, the average of SST of JL1 was 80.70%, significantly higher than that of MY23, which was 27.34% (Fig. 2A and Table 1). The SST of MY23 was largely reduced under natural low-temperature conditions, and MY23 showed cold sensitivity to cold stress. In contrast, JL1 showed stronger cold tolerance than MY23 at the booting and flowering stage.

Table 1
Phenotypic variation in SST for the their parents and RIL populations grown in Beijing and Songming

Environments	Parents		RIL population	
	SST^{JL1} (%)	SST^{MY23} (%)	Mean (%)	Range (%)
18BJ	93.15	86.86	75.70 ± 12.58	32.69–93.97
19BJ	93.62	70.94	85.07 ± 7.57	49.33–97.50
avSST for BJ	93.39	78.9	80.38 ± 8.29	54.25–94.85
16SM	85.61	33.25	33.11 ± 25.01	0-85.11
17SM	82.45	29.23	20.53 ± 22.71	0-88.84
18SM	74.03	19.52	25.80 ± 23.90	0-90.81
avSST for SM	80.7	27.34	26.49 ± 15.79	0.83–76.78
reSST for SM/BJ	86.41	34.65	33.10 ± 19.35	1.00-88.85

There was great variation of the SST, aSBJ, aSSM and CSTI under different environment (Fig. 2B-C and Table 1). Overall, the SST of BJ envioromnets were better than in SM under low temperature conditions. The ranges of aSBJ were 54.25–94.85%, and 0.83–76.78% for aSSM, and the range of CSTI was 1.00-88.85%. The SST in SM (E1, E2 and E3), aSSM and CSTI were used to QTL mapping for cold tolerance.

Genetic map construction

There were 295 molecular markers showing polymorphisms between the parents and even distribution across the 12 chromosomes. Finally, the genetic linkage map was constructed based on 291 polymorphic markers, which covering of the rice genome 2619.10 cM at an average interval of 9.00 cM (Fig. 3). The longest chromosome was chromosome 1, which was 353.48 cM, with an average interval genetic distance of 12.62 cM per marker. The shortest chromosome was chromosome 12, with a length of 137.48 cM and an average interval of 9.82 cM.

QTL mapping

To estimate the cold tolerance, the SST in SM (E1, E2 and E3), aSSM and CSTI under natural low-temperature conditions were used for QTL mapping. A total of nine QTL for the SST related trait were detected under different environments (Table 2 and Fig. 3). These QTL were distributed on chromosome 1, 2, 3, 5, 7, 11 and 12, with LOD scores ranging from 2.64–4.76, which explain the phenotypic variance explained (PVE) range from 3.34 to 12.02%. Among of these QTL, eight QTL positive additive effect from JL1, *qSST2.1, qSST2.2, qSST2.3, qSST3*, *qSST5*, *qSST7*, *qSST11* and *qSST12*; only one QTL, *qSST1*, positive additive effect from MY23. Four, two and one QTL for E1, E2 and E3 under low natural low-temperature conditions, five and three QTL were detected in the aSSM and CSTI, respectively.

Table 2
QTL analysis of SST for the RIL population under natural low-temperature conditions

QTL	Chr.	Peak position	Interval markers	LOD	PVE(%)	Add	Source of allele
The seed setting rate							
qSST1	1.00	223.00	RM446 - RM488	3.37	5.74	-6.15	MY23
qSST2.1	2.00	16.00	ID2000007 - RM279	2.75	6.05	5.17	JL1
qSST2.3	2.00	243.00	AE02004877 - RM250	3.56	7.60	6.97	JL1
qSST3	3.00	16.00	AH03000403 - ID3003462	3.18	5.83	6.10	JL1
qSST5	5.00	150.00	CMB0526.3 - ID5014265	3.48	8.67	6.19	JL1
qSST11	11.00	73.00	RM536 - ID11004341	3.10	5.28	6.05	JL1
qSST12	12.00	43.00	S12011B - RM277	4.21	4.53	19.88	JL1
The average SST for Songming							
qSST1	1.00	219.00	RM446 - RM488	3.09	6.05	-4.26	MY23
qSST2.2	2.00	141.00	S02057B - RM341	2.64	3.62	3.26	JL1
qSST5	5.00	152.00	CMB0526.3 - ID5014265	3.75	5.18	3.90	JL1
qSST7	7.00	197.00	RM21810 - SLG7-GC	2.73	4.19	3.51	JL1
qSST12	12.00	31.00	S12011B - RM277	3.47	8.92	5.95	JL1
Cold stress tolerance index							
qSST1	1.00	218.00	RM446 - RM488	3.25	4.23	-5.79	MY23
qSST5	5.00	152.00	CMB0526.3 - ID5014265	3.81	3.34	5.11	JL1
qSST12	12.00	35.00	S12011B - RM277	4.76	12.02	10.71	JL1
Add represent for additive effect.							

The *qSST1*, *qSST5* and *qSST12* were consistently identified in different conditions, which was considered stable expression QTL. The major QTL *qSST1*, in which the positive additive effect was from MY23, was detected on the chromosome 1 between RM446 and RM488 associated with the SST of E1, aSSM and CSTI. Two major QTL *qSST5* and *qSST12*, in which the positive additive effect was from JL1, were identified on chromosome 5 (CMB0526.3-ID5014265) and on chromosome 12 (S12011B-RM277), respectively. Among these QTL, *qSST12* had the highest LOD value and PVE, which were 4.76 and 12.02%, respectively.

QTL for SST by environmental interactions

To some extent, genotype environment interaction may play an important role in determining rice cold resistance. Thus, the effects and contributions of QTL-by-environment for rice cold tolerance under three years conditions were examined in this study. Thirteen environmental interactions QTL were detected on chromosomes 1, 2, 3, 4, 5, 7, 8, 10, 11 and 12 under three natural low-temperature conditions (Table 3). The QTL *qeSST4*, *qeSST8.1, qeSST8.2* and *qeSST10* were detected only with environmental effects, and the others QTL *qeSST1, qeSST2.1, qeSST2.2, qeSST2.3, qeSST3, qeSST5, qeSST7, qeSST11* and *qeSST12* co-localized with the additive effect QTL as shown in Table 2. Among of these QTL, *qeSST2.1*, *qeSST3, qeSST8.1* and *qeSST10* have a large environmental effect values, the others QTL *qeSST2.2*, *qeSST4* and *qeSST12* environmental effect relatively smaller. These results indicating that environmental interaction QTL played a important role in explaining SST phenotypic variation under natural low-temperature conditions.

Table 3
QTL analysis of SST for the RIL populations by environmental interactions under natural low-temperature conditions

QTL	Chr.	Peak position	Interval markers	LOD	PVE	Add	AE01	AE02	AE03
qeSST1	1	223	RM446 - RM488	4.62	3.81	-3.57	-2.37	0.46	1.91
qeSST2.1	2	16	ID2000007 - RM279	2.78	2.22	1.93	-1.87	3.24	-1.37
qeSST2.2	2	140	S02057B - RM341	2.91	2.37	3.09	-0.27	0.38	-0.11
qeSST2.3	2	246	AE02004877 - RM250	4.73	3.76	2.91	2.90	0.52	-3.42
qeSST3	3	17	AH03000403 - ID3003462	3.94	3.09	3.01	2.48	-0.44	-2.04
*qeSST4**	4	160	S04097A - S04107	2.65	2.03	2.84	0.26	0.29	-0.56
qeSST5	5	151	CMB0526.3 - ID5014265	4.04	3.14	3.04	-1.01	2.63	-1.62
qeSST7	7	199	RM21810 - SLG7-GC	3.56	2.57	2.51	1.18	1.68	-2.86
*qeSST8.1**	8	103	CMB0805.6 - S08052C	2.66	2.18	-1.01	-1.37	-2.53	3.90
*qeSST8.2**	8	219	GW8-AG - S08121A	2.53	2.05	-2.54	-1.67	1.68	-0.02
*qeSST10**	10	0	RM25673 - RM24932	3.92	3.06	2.60	1.49	1.88	-3.37
qeSST11	11	72	RM536 - ID11004341	3.52	2.98	3.02	2.79	-1.36	-1.43
qeSST12	12	26	S12011B - RM277	3.56	2.82	4.26	-0.47	-0.18	0.66

*QTL detected only with environmental effects; AE represents for the predicted additive by environment interactive effect, AE01, AE02 and AE03 represent for 2016, 2017 and 2018, respectively.

Discussion

Phenotypic variation under natural low-temperature conditions

Low-temperature at the booting and heading stage is a serious abiotic stress in rice, and cold tolerance is a complex trait controlled by many quantitative trait loci and environment factors. Most QTL for cold

tolerance at the booting and heading stage have been detected by deep cold-water irrigation and artificial chamber environments (Saito et al. 2001; Endo et al. 2016; Sun et al. 2018). Compared to the cold-water irrigation and growth chamber method, natural low-temperature treatment method for temperature control is relatively simple and more effective treatment and it is suitable for mass verification (Xu et al. 2008). In addition, the phenotypic identification and founded out QTL through the natural low-temperature treatment can be directly applied to production. Yunnan province, is suit for rice natural low-temperature stress treatment, due to its a high altitudeis and climatic conditions which bring about low-temperatures or cold environment for each growth and development periods, especially for the booting and flowering stage in rice. Therefore, many previously studies of cold tolerance experiments have been carried out in Yunnan (Jiang et al. 2010, 2011; Xu et al. 2008; Zhou et al. 2010). Zhu et al. (2015) identified six QTL for cold tolerance on the chromosomes 3, 4 and 12 at the booting stage under natural low-temperature condition by association analysis. The major QTL *qCTB7* associated with cold tolerance under Yunnan natural low-temperature environment, was detected on the chromosome 7 between markers RI02905 and RM21862, is about to 92-kb contains 12 putative candidate gene (Zhou et al. 2010). In previously study, we selected a strong cold resistance *japonica* rice varieties JL1, and a cold-sensitive i*ndica* rice variety MY23 as the parents and developed to the $F_{2,}$ $F_{2:3}$ and RIL populations, which the values of plant height, panicle length, panicle extraction and the SST were largely reduced in natural low-temperature environments (data not shown), these results indicating that cold stress has a major impact on the phenotype of rice(Han et al. 2005c). In this study, the RIL populations from the JL1 and MY23 was used to detected phenotype and QTL under natural low-temperature condition at muti-environments. The lower temperature have a significant effect on SST at three years, the SST, and CSTI traits of the RIL varied exhibited an approximate continuous normal distribution, meanwhile, the transgressive segregation that fell beyond the parents was observed. There have 22 QTL was detected under different environments, nine addictive QTL and thirteen environment interaction QTL for cold resistance. These result suggested that natural low-temperature condition is help to identify cold resistance and founded out novel QTL.

Comparison with previous studies for QTL confer cold tolerance

Rice is a cold-sensitive crop, low-temperature have influences on multiple stages of growth and development in rice, such as germination, and seedlings exposed to low temperatures exhibit slow development, reduced tillers, yellowish leaves and rot (Andaya and Mackill 2003a). The booting and flowering stage of rice is the most sensitive period for cold stress, which affects the development of pollen, the setting rate, and then ultimately the yield. In this study, nine QTL related to cold resistance were detected, *qSST1*, *qSST5* and *qSST12* were considered stable expression QTL. A major QTL *qSST12* was identified on the chromosome 12, which expressed at different environment and have a highest LOD and PVE. Comparison with previous studies revealed that in this region there has been mapped to many QTL related to cold resistance. Among them, *qLTG-12*, *qLTG12a*, *qLTG12b, qLTG12c* and *qLTG12a* were determined for low-temperature germinability (Li et al. 2013; Fujino et al. 2015; Jiang et al. 2020)).

qCTS12a and *qCTS-12* was related to cold tolerance at the seedling stage (Andaya and Mackill 2003a; Zhang et al. 2013). *qCTB12* was related to cold tolerance and was detected in RM292-RM260 on chromosome 12 at the booting stage by Andaya and Mackill (Andaya and Mackill 2003b).

The major *qSST5* was detected on chromosome 5 between the CMB0526.3 and ID5014265, were consistently identified in E2, aSSM and CSTI. Comparison with previous studies revealed that this region has been mapped to many related QTL (Fig. 4). Among these QTL, *qSV-5c* was related to seed vigor at the germination stage, has been mapped to 400-kb genomic region on the chromosome 5 (Xie et al. 2014). Association mapping based on 5K rice array of 249 *indica* rice varieties widely distributed in China determined the QTL *qCTSR5-2* using severity of damage and seed survival rate as the cold-tolerant indices (Zhang et al. 2018). $qLTG(I)_5$, $qLTG(II)_5$ and $qLTGS(I)_5$ was detected on the chromosomes 5 associated with low-temperature germination (LTG) and low-temperature stress index (Najeeb et al. 2020). *qCST5* affecting the cold tolerance at seedling stage was identified from a cold-tolerant variety IL112 (Liu et al. 2013). Yang et al. (2013) adopted NGS-assisted BSA QTL method conferring cold tolerance at the seedling stage in rice, *qCTSS-5* has been mapped on the chromosomes 5 range from 25.40 to 29.63 Mb. *OsRAN2* associated with cold tolerance of the seedling stage is nearby this region (Zang et al. 2010). *qCTB5, qCTB-5-1* and *qCTB-5-2* is related to cold tolerance and has been detected on chromosome 5 at the booting stage (Andaya and Mackill 2003b; Xu et al. 2008). These related QTL were detected at different stages or environment, indicating that the QTL *qSST5* from the chromosome 5 (RM87-CMB0526.3) is a major QTL for cold tolerance, and that these QTL locus are likely stable expression at all growth stages. Thus, the *qSST5* real existence and play an key role in enhance cold tolerance in rice, which can be as a candidate QTL to further fine mapping and application breeding new varieties.

Conclusion

To identification QTL related to cold tolerance at the booting and flowering stage in rice, a set of RIL populations were constructed using a cold-tolerant *japonica* cultivated variety JL1 and a cold-sensitive *indica* cultivated variety MY23. The RIL populations and the parental lines were planted under natural low-temperature conditions, and then natural cold stress had a significant influence on the SST related traits of the parents and RIL populations. Nine QTL were detected on chromosome 1, 2, 3, 5, 7, 11 and 12, with LOD values ranging from 2.64–4.76, the phenotypic variance explained by each QTL ranged from 3.34 to 12.02%. Three QTL *qSST1*, *qSST5* and *qSST12* were stable expression under different conditions and were considered stable major QTL. *qSST5* was identified on chromosome 5 between CMB0526.3 and ID5014265, many related QTL from the previous studies have been identified in this region, so that this major QTL was considered as the candidate region to fine mapping and enhanced cold tolerance gene resources at natural low temperature in rice.

Declarations

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Data Availability

All data included in this study are available upon request by contact with the corresponding author.

Animal Research (Ethics)

Not applicable

Consent to Participate (Ethics) Not applicable

Consent to Publish (Ethics)

Not applicable

Plant Reproducibility

Not applicable

Clinical Trials Registration

Not applicable

Author Contribution

Lina Zhang conducted field work, generated phenotypic data, preformed data analysis and wrote the manuscript; Jianghong Tang generated phenotypic data and genotypic data; Di Cui preformed the genotyping of the mapping population; Cuifeng Tang helped for field work; Xiaoding Ma helped for field work; Xinxiang A helped for field work; Bing Han helped for field work; Guilan Cao helped for field work; Zhengwu Zhao designed the research and manuscript revision; Hee-Jong Koh designed the research and

manuscript revision; Longzhi Han conceived the experiment, guided experiments and manuscript revision. All authors read and approved the final version.

Conflict of Interest

The authors declare that they have no competing interests.

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References

1. Andaya VC, Mackill DJ (2003a) Mapping of QTLs associated with cold tolerance during the vegetative stage in rice. J Exp Bot 54 (392):2579-2585. doi:10.1093/jxb/erg243
2. Andaya VC, Mackill DJ (2003b) QTLs conferring cold tolerance at the booting stage of rice using recombinant inbred lines from a *japonica* x *indica* cross. Theor Appl Genet 106 (6):1084-1090. doi:10.1007/s00122-002-1126-7
3. Cruz RPd, Sperotto RA, Cargnelutti D, Adamski JM, FreitasTerra TD, Fett JP (2013) Avoiding damage and achieving cold tolerance in rice plants. Food Energy Secur 2 (2):96-119. doi:10.1002/fes3.25
4. Endo T, Chiba B, Wagatsuma K, Saeki K, Ando T, Shomura A, Mizubayashi T, Ueda T, Yamamoto T, Nishio T (2016) Detection of QTLs for cold tolerance of rice cultivar 'Kuchum' and effect of QTL pyramiding. Theor Appl Genet 129(3):631–640. doi: 10.1007/S00122-015-2654-2
5. Fujino K, Obara M, Shimizu T, Koyanagi KO, Ikegaya T (2015) Genome-wide association mapping focusing on a rice population derived from rice breeding programs in a region. Breed Sci 65 (5):403-410. doi:10.1270/jsbbs.65.403
6. Han LZ, Zhang SY, Qiao YL, Ruan RC, Zhang JG, Cao GL, Koh HJ (2005a) QTL analysis of root traits at the seedling stage in rice under cold water irrigation. Acta Agronomica Sinica 31(11):1415-1421 (in Chinese)
7. Han LZ, Qiao YL, Zhang YY, Cao GL, Yea JD, Koh HJ (2005b) Identification of QTLs for cold tolerance at the booting stage in rice. Acta Agronomica Sinica 31(5):653-657 (in Chinese)
8. Han LZ, Qiao YL, Zhang SY, Cao GL, Ye CR, Xu FR, Dai LY, Ye JD, Koh HJ (2005c) QTL analysis of some agronomic traits in rice under different growing environments. Scientia Agricultura Sinica

38(6):1080-1087 (in Chinese)

9. Jiang S, Yang C, Xu Q, Wang L, Yang X, Song X, Wang J, Zhang X, Li B, Li H, Li Z, Li W (2020) Genetic dissection of germinability under low temperature by building a resequencing linkage map in *japonica* rice. Int J Mol Sci 21 (4). doi:10.3390/ijms21041284
10. Jiang WZ, Lee JH, Chu SH, Ham TH, Woo MO, Cho YI, Chin JY, Han LZ, Xuan YS, Yuan DL, Xu FR, Dai LY, Yea JD, Koh HJ (2010) Genotype×environment interactions for chilling tolerance of rice recombinant inbred lines under different low temperature environments. Field Crops Res 117(2):226–236. doi: 10.1016/J.FCR.2010.03.007
11. Jiang WZ, Jin YM, Lee JH, Lee KL, Piao RH, Han LZ, Shin JC, Jin RD, Cao TH, Pan HY, Du XL, Koh HJ (2011) Quantitative trait loci for cold tolerance of rice recombinant inbred lines in low temperature environments. Mol Cells 32(6):579–587. doi: 10.1007/S10059-011-0186-4
12. Kuroki M, Saito K, Matsuba S, Yokogami N, Shimizu H, Ando I, Sato Y (2007) A quantitative trait locus for cold tolerance at the booting stage on rice chromosome 8. Theor Appl Genet 115(5):593–600. doi: 10.1007/S00122-007-0589-Y
13. Li J, Pan Y, Guo H, Zhou L, Yang S, Zhang Z, Yang J, Zhang H, Li J, Zeng Y, Li Z (2017) Fine mapping of QTL *qCTB10-2* that confers cold tolerance at the booting stage in rice. Theor Appl Genet 131 (1):157-166. doi:10.1007/s00122-017-2992-3
14. Li L, Liu X, Xie K, Wang Y, Liu F, Lin Q, Wang W, Yang C, Lu B, Liu S, Chen L, Jiang L, Wan J (2013) qLTG-9, a stable quantitative trait locus for low-temperature germination in rice (*Oryza sativa* L.). Theor Appl Genet 126 (9):2313-2322. doi:10.1007/s00122-013-2137-2
15. Liu FX, Xu WY, Song Q, Tan LB, Liu JY, Zhu ZF, Fu YC, Su Z, Sun CQ (2013) Microarray-assisted fine-mapping of quantitative trait loci for cold tolerance in rice. Mol Plant 6 (3):757–767. doi: 10.1093/MP/SSS161
16. McCouch SR, Cho YG, Yano M, Paul E, Blinstrub M, Morishima H, Kinoshita T (1997) Report on QTL nomenclature. Rice Genet Newsl 14:11–13
17. Najeeb S, Ali J, Mahender A, Pang YL, Zilhas J, Murugaiyan V, Vemireddy LR, Li Z (2020) Identification of main-effect quantitative trait loci (QTLs) for low-temperature stress tolerance germination- and early seedling vigor-related traits in rice (*Oryza sativa* L.). Mol Breeding 40 (1): 10. doi:10.1007/S11032-019-1090-4
18. Saito K, Miura K, Nagano K, Hayano-Saito Y, Araki H, Kato A (2001) Identification of two closely linked quantitative trait loci for cold tolerance on chromosome 4 of rice and their association with anther length. Theor Appl Genet 103:862-868. doi:10.1007/s001220100661
19. Saito K, Hayano-Saito Y, Kuroki M, Sato Y (2010) Map-based cloning of the rice cold tolerance gene *Ctb1*. Plant Sci 179 (1-2):97-102. doi:10.1016/j.plantsci.2010.04.004
20. Saito K, Hayano-Saito Y, Maruyama-Funatsuki W, Sato Y, Kato A (2004) Physical mapping and putative candidate gene identification of a quantitative trait locus *Ctb1* for cold tolerance at the booting stage of rice. Theor Appl Genet 109 (3):515-522. doi:10.1007/s00122-004-1667-z

21. Sasaki T, Burr B (2000) International rice genome sequencing project: the effort to completely sequence the rice genome. Curr Opin Plant Biol 3: 138-141. doi: 10.1016/S1369-5266(99)00047-3
22. Sun J, Yang L, Wang J, Liu H, Zheng H, Xie D, Zhang M, Feng M, Jia Y, Zhao H, Zou D (2018) Identification of a cold-tolerant locus in rice (Oryza sativa L.) using bulked segregant analysis with a next-generation sequencing strategy. Rice (N Y) 11 (1):24. doi:10.1186/s12284-018-0218-1
23. Ooijen JV, Ooijen JV, Verlaat JV, Ooijen J, Tol JV, Dalen J, Buren J, Meer JVD, Krieken JV, Ooijen J, Kessel JV, Van O, Voorrips R, Heuvel LVD (2006) JoinMap® 4, Software for the calculation of genetic linkage maps in experimental populations. Kyazma B.V., Wageningen, Netherlands
24. Tang JH, Ma XD, Cui D, Han B, Geng LY, Zhao ZW, Li YF, Han LZ (2019) QTL analysis of main agronomic traits in rice under low temperature stress. Euphytica 215 (12). doi:10.1007/s10681-019-2507-1
25. Voorrips RE (2002) MapChart: software for the graphical presentation of linkage maps and qtls the journal of heredity 93(1):77-78
26. Xie, LX, Tan ZW, Zhou YY, Xu RB, Feng LB, Xing YZ, Qi XQ (2014) Identification and fine mapping of quantitative trait loci for seed vigor in germination and seedling establishment in rice. J Integr Plant Biol 56 (8):749–759. doi:10.1111/JIPB.12190
27. Xu FR, Yu TQ, Tang CF, A XX, Fan CZ, Hu YL, Zhang DY, Dong C, Dai LY (2008) Low-temperature response to major agronomic traits by using recombinant inbred line (RIL) populations derived from Towada/Kunmingxiaobaigu. Scientia Agricultura Sinica 41(11):3437-3447 (in Chinese)
28. Xu LM, Zhou L, Zeng YW, Wang FM, Zhang HL, Shen SQ, Li ZC (2008) Identification and mapping of quantitative trait loci for cold tolerance at the booting stage in a *japonica* rice near-isogenic line. Plant Sci 174 (3):340-347. doi:10.1016/j.plantsci.2007.12.003
29. Yang, ZM, Huang DQ, Tang WQ, Zheng Y, Liang KJ, Cutler AJ, Wu WR (2013) Mapping of quantitative trait loci underlying cold tolerance in rice seedlings via high-throughput sequencing of pooled extremes. PLOS ONE 8 (7). doi: 10.1371/JOURNAL.PONE.0068433
30. Zang AP, Xu XJ, Neill S, Cai WM (2010) Overexpression of *OsRAN2* in rice and Arabidopsis renders transgenic plants hypersensitive to salinity and osmotic stress. J Exp Bot 61(3): 777–789. doi: 10.1093/JXB/ERP341
31. Zeng YW, Yang SM, Cui H, Yang XJ, Xu LM, Du J, Pu XY, Li ZC, Cheng ZQ, Huang XQ (2009) QTLs of cold tolerance-related traits at the booting Stage for NIL-RILs in rice revealed by SSR. Genes Genom 31 (2):143-154. doi:Doi 10.1007/Bf03191147
32. Zhang S, Zheng J, Liu B, Peng S, Leung H, Zhao J, Wang X, Yang T, Huang Z (2013) Identification of QTLs for cold tolerance at seedling stage in rice (*Oryza sativa* L.) using two distinct methods of cold treatment. Euphytica 195 (1):95-104. doi:10.1007/s10681-013-0977-0
33. Zhou L, Zeng YW, Zheng WW, Tang B, Yang SM, Zhang HL, Li JJ, Li ZC (2010) Fine mapping a QTL *qCTB7* for cold tolerance at the booting stage on rice chromosome 7 using a near-isogenic line. Theor Appl Gene 121(5): 895–905. doi:10.1007/s00122-010-1358-x

34. Zhang, MC, Ye J, Xu Q, Feng Y, Yuan XP, Yu HY, Wang YP, Wei XH, Yang YL (2018) Genome-wide association study of cold tolerance of Chinese *indica* rice varieties at the bud burst stage. Plant Cell Rep 37 (3): 529–539. doi: 10.1007/S00299-017-2247-4
35. Zhang Z, Li J, Pan Y, Li J, Zhou L, Shi H, Zeng Y, Guo H, Yang S, Zheng W, Yu J, Sun X, Li G, Ding Y, Ma L, Shen S, Dai L, Zhang H, Yang S, Guo Y, Li Z (2017) Natural variation in *CTB4a* enhances rice adaptation to cold habitats. Nat Commun 8:14788. doi:10.1038/ncomms14788
36. Zhu YJ, Chen K, Mi XF, Chen TX, Ali J, Ye GY, Xu JL, Li ZK (2015) Identification and fine mapping of a stably expressed QTL for cold tolerance at the booting stage using an interconnected breeding population in rice. PLoS One 10(12): e0145704

Figures

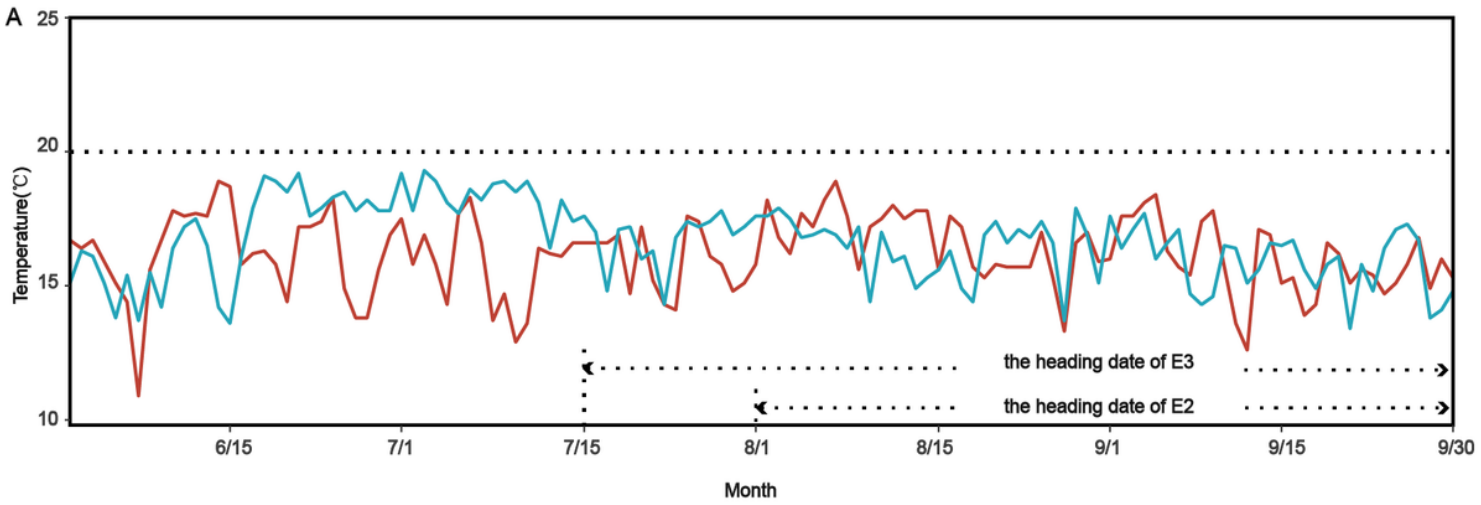

Figure 1

Temperature records for Songming in 2017 (red) and 2018 (blue). The horizontal dotted line represents 20°C, and below the horizontal dotted line are the daily minimum temperatures, respectively. Bottom right of the graph show the heading date of E2 and E3.

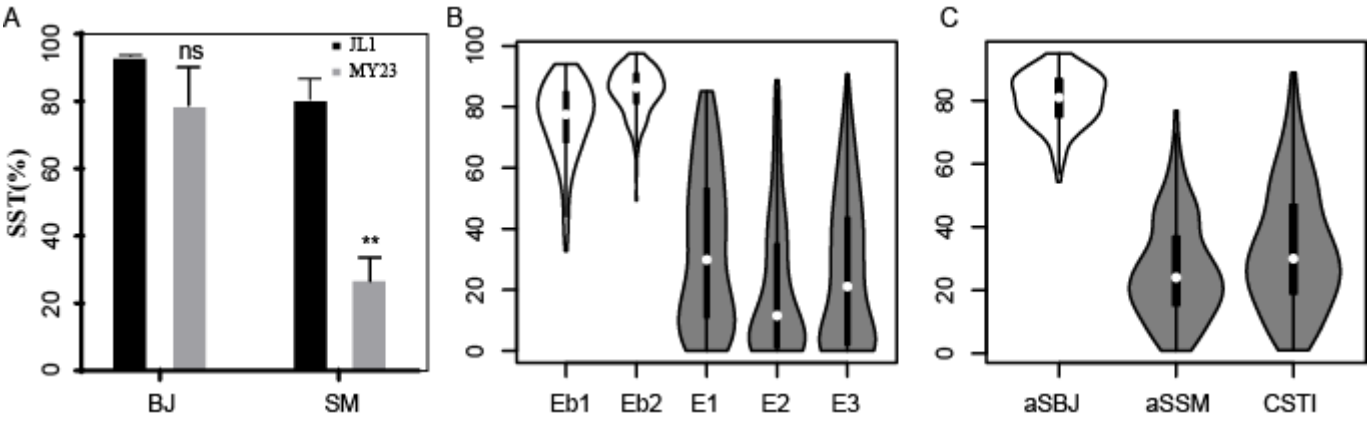

Figure 2

The SST of parents and RIL population in Beijing and Songming. A The statistical comparison of the mean SST of parents planted in Beijing (BJ) and Songming (SM). B Violin plots of the frequency distributions of SST for the RIL population grown in Beijing and Songming. C Violin plots of the frequency distributions of aSBJ, aSSM and CSTI.

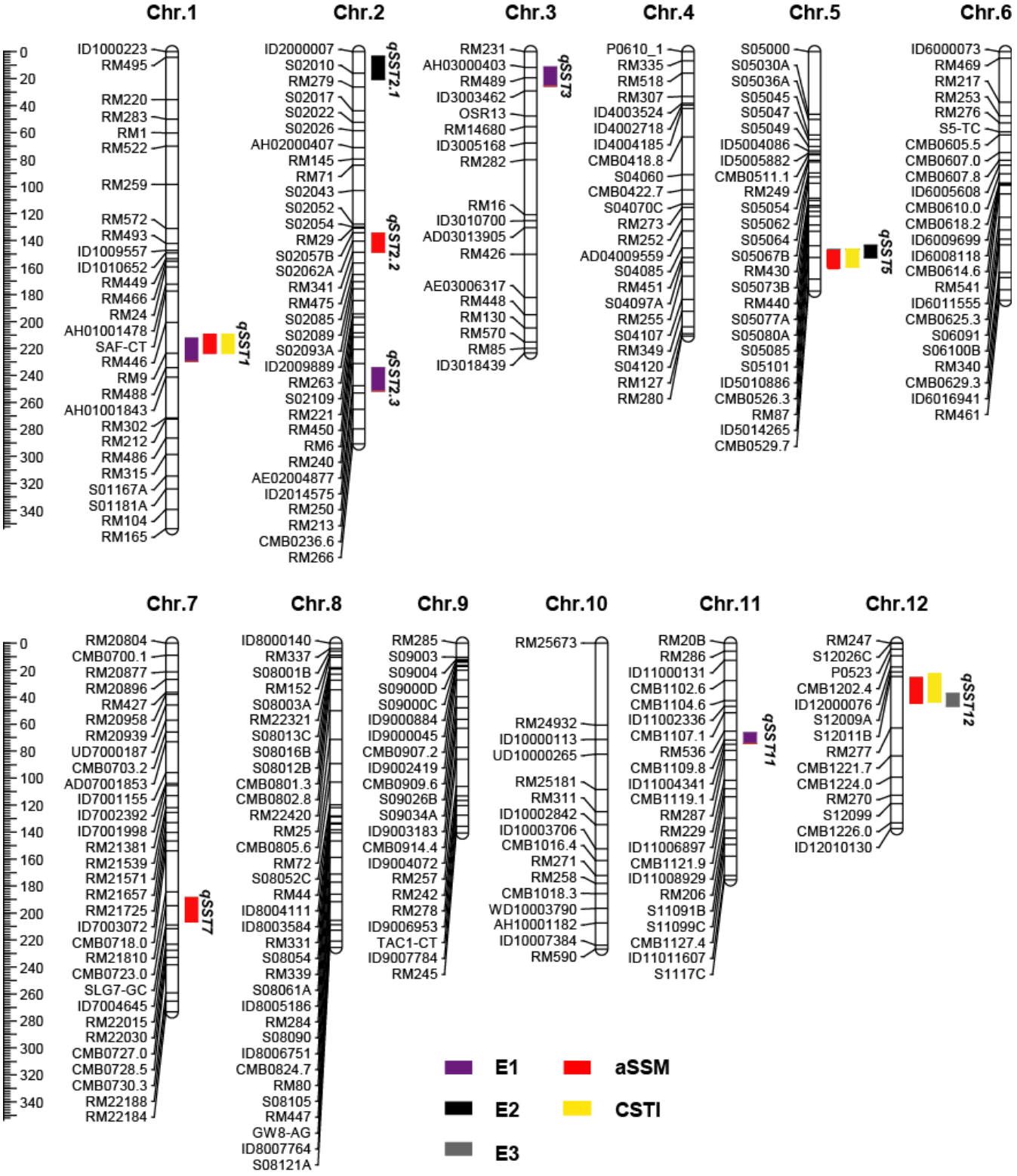

Figure 3

Genetic linkage map of RIL, with map positions of QTL for the SST under natural low-temperature conditions.

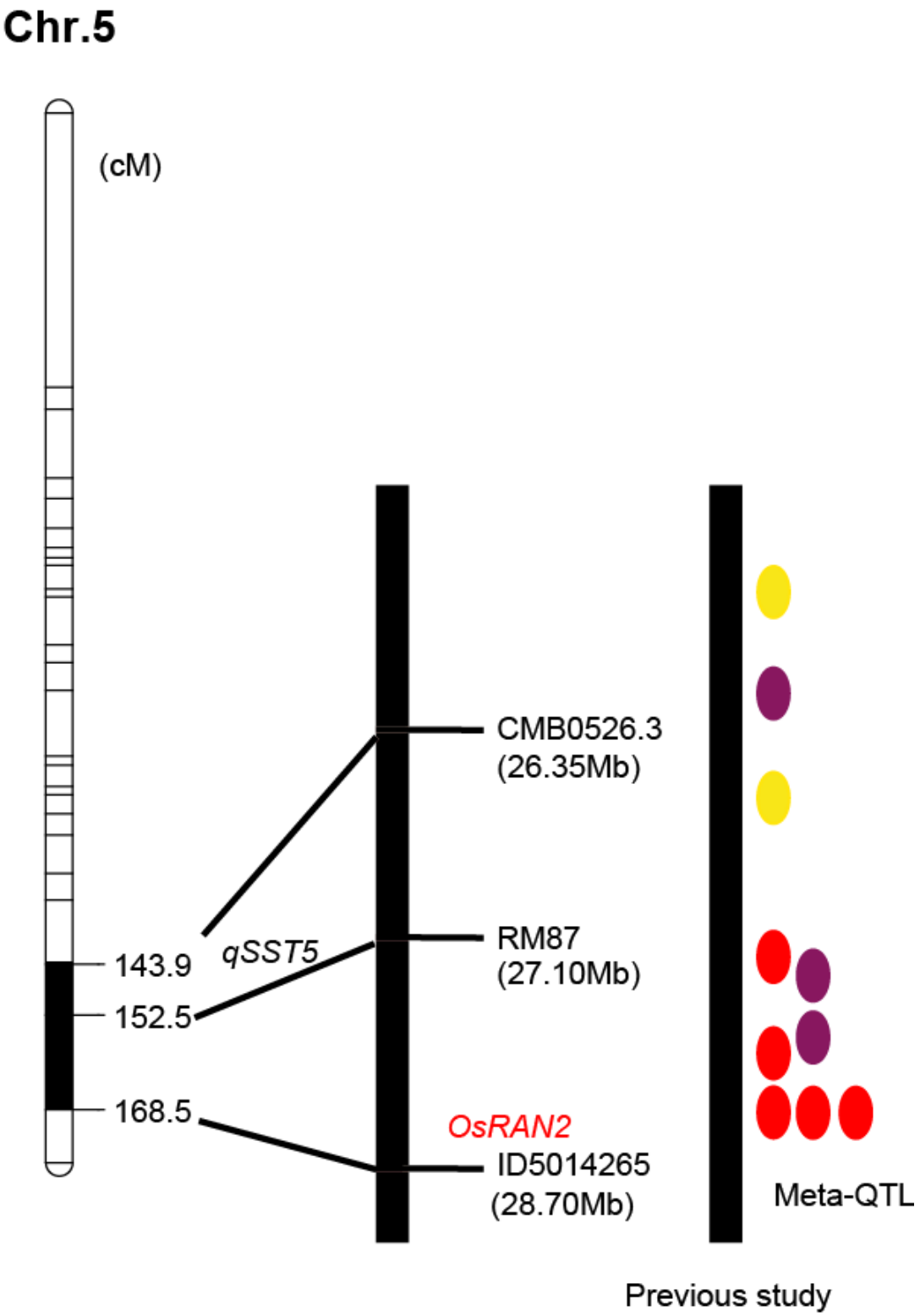

Figure 4

Meta-analysis of QTL confer cold resistant on the chromosome 5. Red circles: QTL for low-temperature germination reported by Xie et al. 2014, Zhang et al. 2018 and Najeeb et al. 2020. Yellow circles: QTL for

cold tolerance at the seedling stage reported by Liu et al. 2013 and Yang et al. 2013. Violet circles: the QTL for cold tolerance at the booting stage reported by Andaya and Mackill 2003b and Xu et al. 2007.