REVIEW

QTL analysis of main agronomic traits in rice under low temperature stress

**Jianghong Tang · Xiaoding Ma · Di Cui · Bing Han · Leiyue Geng ·
Zhengwu Zhao · Yafei Li · Longzhi Han**

Abstract To identify cold tolerance at the reproductive stage in rice (*Oryza sativa* L.), the cold susceptible accession, IAPAR-9 (*tropical japonica*) was crossed with the cold tolerant accessions Akihikari and Liaoyan241 (*temperate japonica*) to construct two recombinant inbred lines (RIL) to detected QTLs related to cold-tolerance of rice through analyze phenotypic variation. The two RILs and their parents were experimentally grown in natural conditions and cold water irrigation at one site, Gongzhuling Jilin (China), and at natural low temperature in another site, Kunming Yunnan (China). We measured plant height, panicle length, number of panicles per plant, grain number per panicle, seed setting rate and panicle exsertion of parents and RIL populations in the maturation stage. In the three treatment environments, the traits of the two RILs showed normal distributions, and both exhibited transgressive segregation. Low temperature stress had greater influence on number of panicles per plant and grain number per panicle and lower influence on plant height and panicle length. Seventeen quantitative trait locus (QTLs) were detected, distributed on chromosomes 1, 3-6, 8, 11 and 12. Among these, four QTLs had values of phenotypic variance explained that were greater than 10%. There were two QTLs, $qPN12\text{-}2^{E2}$ and $qPN12\text{-}2^{E3}$, detected in the IAPAR-9/Akihikari RIL population in same region under cold water irrigation condition in Gongzhuling Jilin and natural low temperature in Kunming Yunnan. Major regions related to cold tolerance QTLs in these two populations was on chromosome 12 from RM511 to RM3739. The cold tolerance allele was from the Akihikari and Liaoyan241 in the respective populations for both QTL regions.

J. Tang · X. Ma · D. Cui · B. Han · L. Geng ·
L. Han (✉)
Institute of Crop Sciences, Chinese Academy of
Agricultural Sciences, Beijing 100081, China
e-mail: hanlongzhi@caas.cn

J. Tang
e-mail: 1274605413@qq.com

J. Tang · Z. Zhao (✉)
Chongqing Engineering Research Center of Specialty
Crop Resource, Chongqing Normal University,
Chongqing 401331, China
e-mail: 1297274357@qq.com

Y. Li (✉)
Institute of Genetics and Developmental Biology, Chinese
Academy of Sciences, Beijing 100101, China
e-mail: yf8811@163.com

Introduction

Rice is a tropical and subtropical crop, sensitive to low temperatures. It is commonly divided into two types: *indica* (*Oryza sativa* subsp. xian) and *japonica* (*Oryza sativa* subsp. geng). Chilling damage can affect all growth stages of rice from reduced survival rate at the seedling stage and reduced percent seed-setting at the booting stage. Chilling damage in rice due to low temperature is a major problem from northern to southern China (Dai et al. 2002; Han et al. 2002; Xiong et al. 1990). A previous study of rice yields in the Liangshan area of the Sichuan Province of China from 2000 through 2016 found 13 instances of chilling damage at various temperatures. Decrease of 1 °C in the average temperature during the booting stage reduces the rice yield by 1029 kg/hm^3 (Cao et al. 2017). In the Anhui Province of China, different degrees of chilling damage were observed from 2002 to 2014 (Wu et al. 2017). From 1981 to 2010, low-temperature chilling damage on rice yield in a single season of rice production in China's Jiangsu Province caused 28 instances of moderate cold damage and 18 instances of severe cold damage (Yin et al. 2016). In recent years, the challenges presented by chilling damage in the three northeastern provinces of China have increased significantly (Feng et al. 2013). It is estimated that low temperature chilling damage reduces rice yield by 5–10 billion kg every year in China (Song et al. 2016). Therefore, improving the cold tolerance of rice is an important goal for rice breeders and identifying markers associated with cold tolerance are necessary to expedite breeding efforts.

In the past 20 years, many scholars have focused on conducting genetic studies of cold tolerance in rice. So far, more than 250 QTLs related to cold tolerance at different growth stages of rice have been detected in both biparental and multi-parent populations (Liang et al. 2018). Yang et al. (2015) showed that cold tolerance QTLs were distributed on all 12 rice chromosomes, with an average of 16 QTLs per chromosome. Among which, 45 QTLs were related to the reproductive stage, and most of these were located on chromosome 4. Pan et al. (2015) detected 51 cold-tolerant QTLs in all growth stages of rice, distributed on all 12 chromosomes, and 33 of these QTLs were detected at the booting stage. Zeng et al. (2009) used 10 agronomic traits to detect eight QTLs related to cold tolerance on chromosomes 1, 4 and 5. In five different environments, 58 QTLs were detected in a RIL population constructed by Shihetian and Xiaobaigu, of which 17 QTLs were detected in two or more environments (Zhang et al. 2015). Many QTLs (genes) that associated with cold tolerance of rice at different growth stages have been studied by many researchers, but only a few cold-tolerant QTLs have been fine-mapped and cloned. *Cb1* and *CTB4a* are two of these cold-tolerant QTLs that are associated with the booting stage of rice, and which have been cloned. All of the cold-tolerant QTLs that have been fine-mapped and cloned are located on chromosome 4. *Ctb1* was the first cloned QTL associated with cold tolerance at the booting stage, where it was fine mapped into a 17 kb area located on the long arm of chromosome 4, and contained two genes, encoding an F-box protein and a ser/thr protein kinase (Saito et al. 2010). *CTB4a*, located in the 56.8 kb region, encoded a conserved leucine-rich repeat receptor-like kinase, four open reading frames (ORF), and one candidate gene (*LOC_Os04g04330*) (Zhang et al. 2017). Four more cold-tolerant QTLs that have been fine mapped and are related to the booting stage of rice are *qCTB7*, *qCTB8*, *qCTB10-2* and *qCT-3-2*. *qCTB7* is located in the 92 kb region on the long arm of chromosome 7 and there are 12 putative candidate genes in the 92 kb region (Zhou et al. 2010). *qCTB8* is located in the 193 kb region of RM5647–PLA61 on chromosome 8 and can explains 26.6% of the agronomic variance (Kuroki et al. 2007). *qCTB10-2* is located in the 132.5 kb region of RM25121–MM0568 on chromosome 10. There are 17 putative candidate genes in the 132.5 kb region, of which four were chilling mutant genes (Li et al. 2018). And a stable QTL, *qCT-3-2*, that could be discovered and fine mapped into the 192.9 kb region on chromosome 3 (Zhu et al. 2015).

Cold tolerance in rice is a complex quantitative trait controlled by multiple genes. Although there are many reports on the cold tolerance QTL in rice, these QTLs have low phenotypic variance explained and are not consistently detected across different environments nor years, and these cold tolerance QTLs from previous research have been scarcely applied in rice breeding. Therefore, it is necessary to explore more cold-tolerant QTLs and to find QTL sites that can be effectively used in cold tolerance breeding of rice. Low temperatures will lead to a shortening of plant height and panicle length, a reduction of the number of grains per panicle, a reduction in panicle exertion, a

delayed heading time and a decreased percent seed set (Han et al. 2002). In this study, two RIL populations, IAPAR-9/Akihikari RIL population and IAPAR-9/Liaoyan241 RIL population, were constructed from the *Japonica* cultivars—IAPAR-9 (cold sensitive), Akihikari and Liaoyan 241 (cold tolerance) for mapping population. The two RIL populations were grown in Gongzhuling Jilin, China and Kunming Yunnan, China. Plant height (PH), panicle length (PL), number of panicles per plant (PN), grain number per panicle (GPP), seed setting rate (SST) and panicle exsertion (PE) were all recorded at the maturation stage. We exposed the rice to different low temperatures to identify and observe responses of agronomic traits combined with an analysis of SSR markers to found one or more QTLs multiple regions that are associated with cold tolerance. Our aims were to provide a scientific basis for fine mapping cold tolerance QTLs related to reproductive stage and breeding rice varieties with stronger cold tolerance.

Materials and methods

Materials

The common cold susceptible parent used to develop the two RIL populations was IAPAR-9, a tropical *japonica* upland rice variety from Brazilian Academy of Agricultural Sciences Rice and beans Research Center of Brazil, Akihikari is a temperate *japonica* lowland rice variety from Japan, and Liaoyan241 is a temperate *japonica* lowland rice variety from the Institute of Saline-Alkali Land Use of Liaoning Province, China. IAPAR-9, Akihikari, and Liaoyan241 were provided by the China National Crop Germplasm Bank in Beijing. The registration numbers of the three germplasms are WD-19081, WD-10974, and ZD-03932, respectively. We obtained two recombinant inbred line F_7 rice populations (IAPAR-9/Akihikari RIL population and IAPAR-9/Liaoyan241 RIL population) for this experiment. IAPAR-9 was crossed with both Akihikari and Liaoyan241 to obtain single F_1 plantsand these F_1 plants were self-pollinated to produce F_2 seeds. These F_7 RIL populations were derived from the F_2 seed by the single seed descent method. IAPAR-9/liaoyan241 RIL had 228 RILs,IAPAR-9/Akihika RIL had 231 RILs.

Methods

Cold water stress test

A cold water stress test was conducted at Rice Research Institute, Jilin Academy of Agricultural Sciences [Gongzhuling, Jinlin, China (43°50′N, 124°82′E)]. The two RIL populations and their parents were sown on 13 April, 2009 and transplanted on 26 May. There were two treatments, a cold water irrigation condition and a control using ambient temperature irrigation ("normal" condition), with two replications per treatment of parents and populations. Rice plants were grown in rows with each row for one family of the population, 12 holes per row, and a single plant per hole. The transplanting standard was 25 cm × 15 cm with two lines. Fertilizer of pure nitrogen was directly applied to transplants at 120 kg/hm² in the field. For the cold water stress, plants were constantly irrigated with 19 °C water for about 30–40 days from 1 July at the beginning of rice heading until all late maturing varieties produced panicles. The cold water depth began to adjust to 20 cm, and then added to 30 cm along with the stem elongation. From the end of August to the middle of October, the whole experimental area was covered by a plastic shed to protect all plants until they reached the heading and maturation stages. We recorded plant height (PH, The distance between the rice plant from the ground to the top of the highest panicle [excluding the length of the awn]), panicle length [PL, The length from the neck-panicle node to the top of the panicle (excluding the length of the awn)], number of panicles per plant (PN, The sum of the panicles per plant), grain number per panicle (GPP, The sum of the number of all grains per panicle) and seed setting rate (SST, The ratio of filled grains and total spikelets per panicle) of parents and individuals of both RIL populations when plants reached the maturity stage for both the control and cold water irrigation treatments. Mean values were calculated from five individuals from each family of two repetitions. We calculated a cold-water response index (CRI) for each trait to represent the relative level of cold resistance. The CRI = the trait value under cold water treatment/the trait value under normal condition.

Natural low temperature stress test

A natural low air temperature test was conducted in the experimental field of Yunnan Academy of Agricultural Sciences (Kunming, Yunnan, China (25°05′N, 102°72′E)). During the booting stage of rice grown in Kunming, early June to late August, the air temperature of Kunming is lower than that of other provinces. The average air temperature is about 20–23 °C during this period, which is an ideal ambient low temperature to test cold tolerance of rice at the booting stage. The two RIL populations and their parents were sown on 31 March, 2009 and transplanted on 9 May, with two repetitions per treatment for each population and parent. Rice plants were grown in rows with each row for one family of the population, 20 holes per row, and a single plant per hole. The transplanting standard was 25 cm × 15 cm with two lines. Fertilizer of pure nitrogen was directly applied to transplants at 120 kg/hm^2 in the field. ALL of these parents and populations with two repetitions per treatment. We recorded plant height (PH), panicle length (PL), number of panicles per plant (PN), grain number per panicle (GPP), seed setting rate (SST) and panicle exsertion (PE, The distance between the flag leaf node and the neck-panicle node. When the distance is negative value, the neck-panicle is all wrapped in the sheath leaf sheath.) of parents and individuals of both RIL populations when plants reached the maturity stage. Mean values of recorded traits were calculated from five individuals from each family of two repetitions.

Construction of molecular marker linkage map and QTL detection

We sampled the flag leaf of the parental line and the two RIL populations at the tillering stage. DNA was extracted using the hexadecyl trimethyl ammonium Bromide (CTAB) method. Using 1000 pairs of SSR primers synthesized in a laboratory, we detected polymorphic markers in the parents. These selected markers were covered as much of the entire genome of rice as possible and were uniformly distributed on 12 chromosomes, the polymorphic markers were screened and the genotypes of individual plants were detected. The RIL of IAPAR-9/Akihikari contained 174 SSR markers, and the RIL of IAPAR-9/Liaoyan241 contained 170 SSR markers. The selected SSR marker was listed in supplemental Table 1. We used ICiMapping4.0 to construct the genetic linkage map and map the QTL. Inclusive composite interval mapping (ICIM) was used to identify and analyze QTLs. The minimal LOD value required to declare a QTL was obtained empirically from 1000 permutation tests, with a walk speed of 1 cM. A LOD threshold of 5.0 for these populations and an experiment-wide significance level of 0.05 were set.
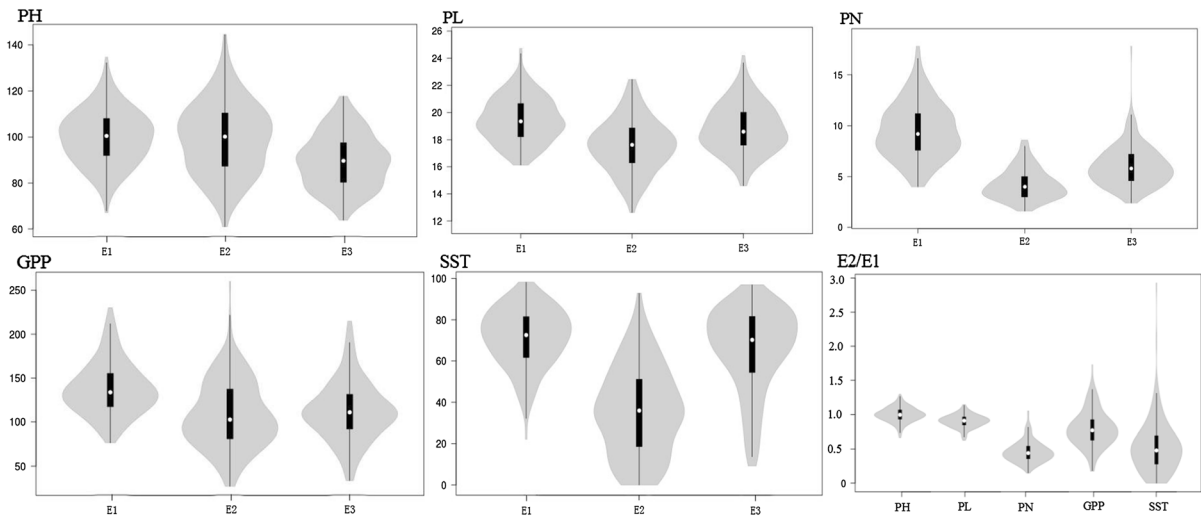
Results

Agronomic traits analysis

Main agronomic traits of IAPAR-9 and Akihikari and their RIL population

The mean values of agronomic traits and their CRI of plants of IAPAR-9, Akihikar and their RIL grown under normal and cold water irrigation conditions in the Gongzhuling Jilin site and natural low temperature in the Kunming Yunnan site are shown in supplemental Table 2 and Fig. 1. All of the agronomic traits of the RIL population in each of the three conditions exhibited transgressive segregation except PN under the Jilin natural condition. The coefficients of variation (CV) were wide ranging in these traits. Of rice plants in the Gongzhuling Jilin normal treatment, the CV of PN was the highest, 28.56%, and the CV of PL was lowest, 8.44%. Under the cold water treatment in Jilin Province, the CV of SST was the highest, 58.92%, and the CV of PL was lowest, 11.09%. Under the natural low temperature condition in Kunming, the CV of PE was the highest, 282.74%, the CV of PL was lowest, 9.51%.

The distribution of agronomic traits in this population was similar to that of a normal distribution; the trait values of most plants fell in the middle and few values were at the two ends of the distribution (Fig. 1). The distributions of the same trait in the IAPAR-9/Akihikari RIL population differed among the three environments; PN differed the most, while PH and PL had the smallest differences. For these traits, a higher CRI is indicative of smaller effects of low temperature. The CRI of PH and PL of the parents and RIL were similar and the highest, the CRI of PN was the lowest, and the CRI of SST was the second from the lowest (supplemental Table 2). Thus, results show that

Fig. 1 Violin plots of frequency distributions for each agronomic trait of the IAPAR-9/Akihikari RIL population grown in three different conditions (Spitzer et al. 2014). *PH* plant height, *PL* panicle length, *PN* number of panicles per plant, *GPP* grain number per panicle, *SST* seed setting rate, *PE* panicle exsertion. E1 is the Gongzhuling Jilin control, E2 is the Gongzhuling Jilin cold treatment, and E3 is the Kunming Yunnan cold treatment, E2/E1 is the ratio of two treatments under Gongzhuling Jilin

cold water stress had little effect on PH and PL, but it had greater effect on PN and SST.

Main agronomic traits of IAPAR-9 and Liaoyan241 and their RIL population

The mean values of agronomic traits and their CRI of plants of IAPAR-9 and Liaoyan241 and their RIL grown under the normal and cold water irrigation conditions in Gongzhuling Jilin and the natural low temperature condition in Kunming is shown in supplemental Table 3 and Fig. 2. All of these agronomic traits of the RIL population under normal and low temperature conditions exhibited transgressive segregation, and the range of the coefficients of variation was wide. The environment has a great influence on the growth of rice, the agronomic traits of IAPAR-9 and Liaoyan241 and their RIL population plants was difference at 3 environments. Of plants in the Gongzhuling Jilin normal treatment, the CV of PN was highest, 30.62%, and the CV of PL was lowest, 7.82%. Of plants in the cold water irrigation treatment in Gongzhuling Jilin, the CV of PSS was highest, 52.44%, and the CV of PL was lowest, 9.83%. In the Kunming natural low temperature treatment, the CV of PE was highest, 772.97%, the CV of PL was lowest, 10.05%. These trait values were normally distributed in the three treatments. The distributions of PN and

SST exhibited the largest difference, and the distribution of PL was similar among plants grown in the three environments. In the IAPAR-9/Liaoyan241 RIL population, the value of CRI of PH and PL were bigger than those of other traits, the value of CRI of PN and SST were small. These results indicate that cold water stress had little influence on PH and PL, but it had greater influence on PN and SST.

Correlations between agronomic traits under low temperature stress

Correlations between agronomic traits under cold water irrigation stress in Gongzhuling, Jilin Province

The correlations between agronomic traits of the two RIL populations grown under cold water irrigation stress in Gongzhuling, Jilin Province are shown in Table 1. In the IAPAR-9/Akihikari RIL population, plant height had an extremely significant positive correlation with both panicle length and seed setting rate. Grain number per panicle showed an extremely significant positive correlation with both panicle length and number of panicles per plant. In the IAPAR-9/Liaoyan241 RIL population, plant height was extremely significantly positively correlated with panicle length and seed setting rate, and negatively with number of panicles per plant. Grain number per
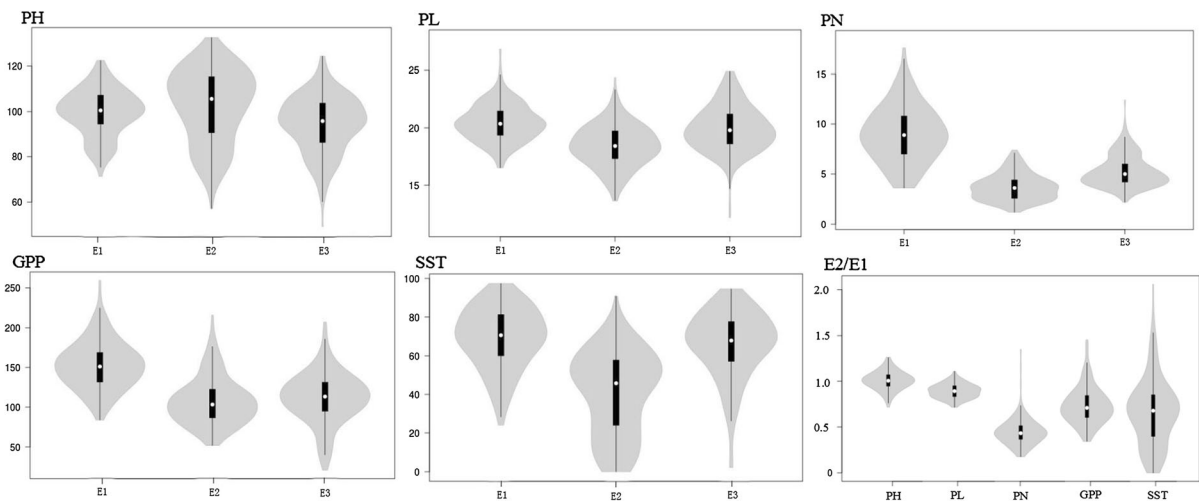
Fig. 2 Violin plots of frequency distributions for each agronomic trait of the IAPAR-9/Liaoyan241 RIL population. *PH* plant height, *PL* panicle length, *PN* number of panicles per plant, *GPP* grain number per panicle, *SST* seed setting rate, *PE* panicle

exsertion. E1 is the Gongzhuling Jilin control, E2 is the Gongzhuling Jilin cold treatment, and E3 is the Kunming Yunnan cold treatment, E2/E1 is the ratio of two treatments under Gongzhuling Jilin

Table 1 Correlations between agronomic traits in two RILs grown under cold water irrigation stress in Gongzhuling of Jilin province

	PH	PL	PN	GPP	SST
PH		0.670**	0.074	0.086	0.290**
PL	0.615**		0.12	0.298**	0.024
PN	− 0.145*	0.077		0.387**	0.036
GPP	0.055	0.352**	0.407**		− 0.106
SST	0.382**	0.113	0.107	0.045	

Data in the top rows of the table show the correlations between traits in the IAPAR-9/Akihikari RIL population; data in lower rows show correlations in the IAPAR-9/Liaoyan241 RIL population

*,** meaned significant at 0.05 and 0.01 levels respectively

PH plant height, *PL* panicle length, *PN* number of panicles per plant, *GPP* grain number per panicle, *SST* seed setting rate, *PE* panicle exsertion

panicle was extremely significantly positively correlated with panicle length and number of panicles per plant.

Correlations between agronomic traits under natural low temperature stress in Kunming, Yunnan Province

Correlations between agronomic traits of the two RIL populations grown under low temperature stress in Kunming, Yunnan Province are shown in Table 2. In

the IAPAR-9/Akihikari RIL population, plant height showed an extremely significant positive correlation with panicle length, grain number per panicle, seed setting rate, and panicle exsertion. Panicle length had an extremely significant positive correlation with number of panicles per plant, grain number per panicle, and panicle exsertion. Number of panicles per plant had an extremely significant positive correlation with grain number per panicle, and strong negative correlation with seed setting rate. Seed setting rate had an extremely significant positive correlation with panicle exsertion. In the IAPAR-9/Liaoyan241 RIL population, plant height had an extremely significant positive correlation with panicle length, grain number per panicle, and panicle exsertion. Panicle length had a significant positive correlation with number of panicles per plant, and an extremely significant positive correlation with grain number per panicle. Panicle exsertion had a significant positive correlation with grain number per panicle, and an extremely significant positive correlation with seed setting rate.

Table 2 Correlations between agronomic traits in two RILs grown under natural low temperature stress conditions in Kunming of Yunnan Province

	PH	PL	PN	GPP	SST	PE
PH		0.584**	0.011	0.197**	0.147**	0.445**
PL	0.271**		0.208**	0.384**	− 0.057	0.188**
PN	− 0.025	0.162*		0.262**	− 0.255**	− 0.126
GPP	0.314**	0.313**	0.122		− 0.062	0.011
SST	0.013	0.043	− 0.109	− 0.004		0.317**
PE	0.528**	0.114	0.074	0.159*	0.224**	

Data in the top rows of the table show the correlations between traits in the IAPAR-9/Akihikari RIL population; data in lower rows show correlations in the IAPAR-9/Liaoyan241 RIL population

*,** meaned significant at 0.05 and 0.01 levels respectively

PH plant height, *PL* panicle length, *PN* number of panicles per plant, *GPP* grain number per panicle, *SST* seed setting rate, *PE* panicle exsertion

QTL identification

QTL mapping of cold tolerance traits of the IAPAR-9/ Akihikari RIL population

The result of QTL mapping of cold tolerance traits of the IAPAR-9/Akihikari RIL population are shown in Table 3 and Fig. 3 under normal and cold water irrigation conditions in Gongzhuling Jilin and the natural low temperature in Kunming. Eleven QTLs were detected in plants grown under the three environments. These QTLs were distributed on chromosomes 1, 3, 5, 6, 11 and 12. The range of phenotypic

variance explained (PVE) of these QTLs was 6.70–12.96%.

Five QTLs were correlated with PH. Four QTLs were correlated with PN, among these, $qPN12\text{-}2^{E2}$ and $qPN12\text{-}2^{E3}$ were both QTLs located in the RM463–RM3739 region on chromosome 12, and were detected under cold water irrigation conditions in Gongzhuling Jilin and under natural low temperatures in Kunming Yunnan, it was same QTL. Two QTLs were correlated with GPP. Of all the QTLs, *qPN12-1*, $qPN12\text{-}2^{E2}$ and $qPN12\text{-}2^{E3}$ had the highest percentages of PVE, at 11.38%, 11.67%, and 12.96%, respectively. And their source of allele was from Akihikari.

Table 3 Summary of QTL detection and genetic effects in IAPAR-9/Akihikari RILs

Traits	Environments	Name	Chr	Range	LOD	PVE (%)	Add	Source of allele
PL	E1	*qPL3*	3	RM5755–RM218	5.03	7.53	− 0.50	Akihikari
		qPL6-1	6	RM20366–RM20512	5.99	6.70	0.48	IAPAR-9
		qPL11	11	RM6091–RM209	5.32	6.70	− 0.48	Akihikari
	E3	*qPL6-2*	6	RM20512–RM1370	5.16	8.86	0.61	IAPAR-9
	E2/E1	*qPL12-1*	12	RM463–RM3739	5.07	7.67	− 2.94	Akihikari
PN	E1	*qPN5*	5	RM87–RM31	5.64	8.95	0.83	IAPAR-9
		qPN12-1	12	RM511–RM463	6.94	11.38	− 0.93	Akihikari
	E2	$qPN12\text{-}2^{E2}$	12	RM463–RM3739	6.06	11.67	− 0.55	Akihikari
	E3	$qPN12\text{-}2^{E3}$	12	RM463–RM3739	7.17	12.96	− 0.71	Akihikari
GPP	E1	*qGPP1-1*	1	RM12137–RM6840	5.02	7.67	− 10.38	Akihikari
	E2/E1	*qGPP12-1*	12	RM463–RM3739	5.04	9.76	− 8.51	Akihikari

E1 is the Gongzhuling Jilin control, E2 is the Gongzhuling Jilin cold treatment, and E3 is the Kunming Yunnan cold treatment, E2/E1 is the ratio of two treatments under Gongzhuling Jilin

PL panicle length, *PN* number of panicles per plant, *GPP* grain number per panicle, *Chr* chromosome
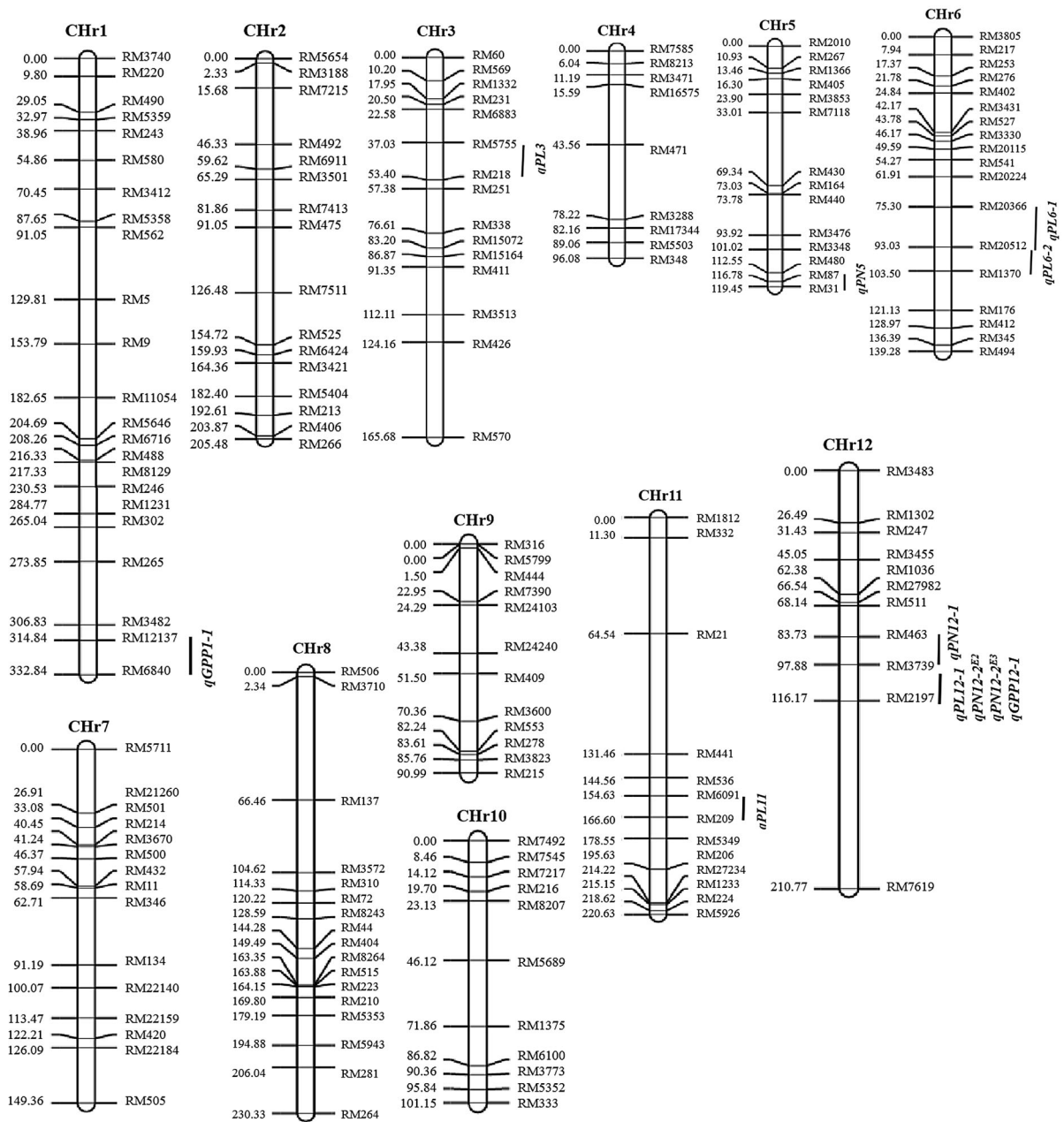
Fig. 3 Interval distribution of QTLs for cold tolerance-related traits in the IAPAR-9/Akihikari RIL population. *PL* panicle length, *PN* number of panicles per plant, *GPP* grain number per panicle

QTL mapping of cold tolerance traits of the IAPAR-9/Liaoyan241 RIL population

The results of QTL mapping of cold tolerance traits of the IAPAR-9/Liaoyan241 RIL population are shown in Table 4 and Fig. 4 under normal and cold water irrigation conditions in Gongzhuling Jilin and the

natural low temperature in Kunming. In total, six QTLs were detected from rice in the three growing conditions. These QTLs were distributed on chromosomes 1, 4, 8 and 12. The range of PVE of these QTLs was 5.97–18.85%.

Two QTLs, *qPH8* and *qPH1*, were related to PH, and their source of allele was from IAPAR-9. One

Table 4 Summary of QTL detection and genetic effects in IAPAR-9/Liaoyan241 RIL population

Traits	Environments	Name	Chr	Range	LOD	PVE (%)	Add	Source of allele
PH	E2	*qPH8*	8	RM44–RM404	6.44	5.97	5.21	IAPAR-9
	E3	*qPH1*	1	RM220–RM490	6.09	9.99	4.61	IAPAR-9
PL	E2	*qPL12-2*	12	RM511–RM463	5.80	8.64	− 0.69	Liaoyan241
PN	E2	*qPN4*	4	RM16575–RM471	8.04	18.85	− 0.49	Liaoyan241
GPP	E2	*qGPP12-2*	12	RM511–RM463	5.10	9.75	− 10.10	Liaoyan241
	E3	*qGPP1-2*	1	RM12137–RM6840	5.80	9.59	− 12.56	Liaoyan241

E2 is the Gongzhuling Jilin cold treatment, E3 is the Kunming Yunnan cold treatment

PH plant height, *PL* panicle length, *PN* number of panicles per plant, *GPP* grain number per panicle, *Chr* chromosome

QTL, *qPL12-2*, was related to PL. One QTL, *qPN4*, were related to PN. Two QTLs, *qGPP12-2* and *qGPP1-2*, were related to GPP. The source of the alleles for the QTLs related to PL, PN, and GPP was Lianyan241. Among these, *qPN4* had the highest percentages of PVE, at 18.85%.

Discussion

Analysis of QTL results for each trait

In cold temperatures stress, percent seed-setting is a more direct measure to identify and evaluate rice cold tolerance at the booting stage of rice than the other agronomic traits, such as PH, PL, PN and GPP, which are indirect measures (Xu et al. 2008). The results of the agronomic analysis shows that all the agronomic traits had different degrees of transgressive segregation and were approximately normally distributed. These traits were quantitative traits controlled by multiple genes, and low temperature had a major effect on PN and SST, but only a minor effect on PH and PL. These results are consistent with results of previous reports (Han et al. 2004; Guo et al. 2003; Jena et al. 2012).

The effect and location of QTLs exhibited by a single population and a single treatment condition are likely only relevant to the respective population or treatment and not across populations and treatments. The QTLs were dissimilar because of dissimilar growth environments and genetic backgrounds. To investigate the stability of QTLs, we detected QTLs in response to multiple cold environments over multiple years in previous studies (Malosetti et al. 2008; Messmer et al. 2009). In this study of two RIL

populations and three rice-growing environments (normal and cold water irrigation conditions in Gongzhuling Jilin and the natural low temperature in Kunming), seventeen QTLs were detected. The range of PVE of these QTLs was 5.97–18.85%. They were distributed on all chromosomes except chromosome 2, 7 and 9. Among them, the PVE of four QTLs were greater than 10%. The PVE of three QTLs that from IAPAR-9/Akihikari RIL population were greater than 10%, PVE of one QTLs that from IAPAR-9/Liaoyan241 RIL population were greater than 10%.

In IAPAR-9/Akihikari RIL population, RM463–RM3739 on chromosome 12 had 4 QTLs, *qPN12-2^{E2}*, *qPN12-2^{E3}*, *qPL12-1* and *qGPP12-1*. Moreover, the source of alleles of these QTLs was from Akihikari. Among these, *qPN12-2^{E2}* and *qPN12-2^{E3}*, from the cold water irrigation treatment in Gongzhuling Jilin and natural low temperature treatment in Kunming, respectively, were associated with number of panicles per plant, they was same QTL. Correlation analysis for these traits showed that under the cold water irrigation conditions in Gongzhiling, Jilin, number of grains per panicle had a significant positive correlation with panicle length and number of panicles per plant. Under the natural low temperature conditions in Kunming, there were significant positive correlations between number of grains per panicle, panicle length, and number of panicles per plant. This suggests that the QTLs responsible for these traits are interacting.

Comparisons with previous studies of chromosomal regions with multiple QTLs

In all regions with multiple QTLs in the RIL population IAPAR-9/Akihikari, the region of RM463–RM3739 on chromosome 12 had multiple
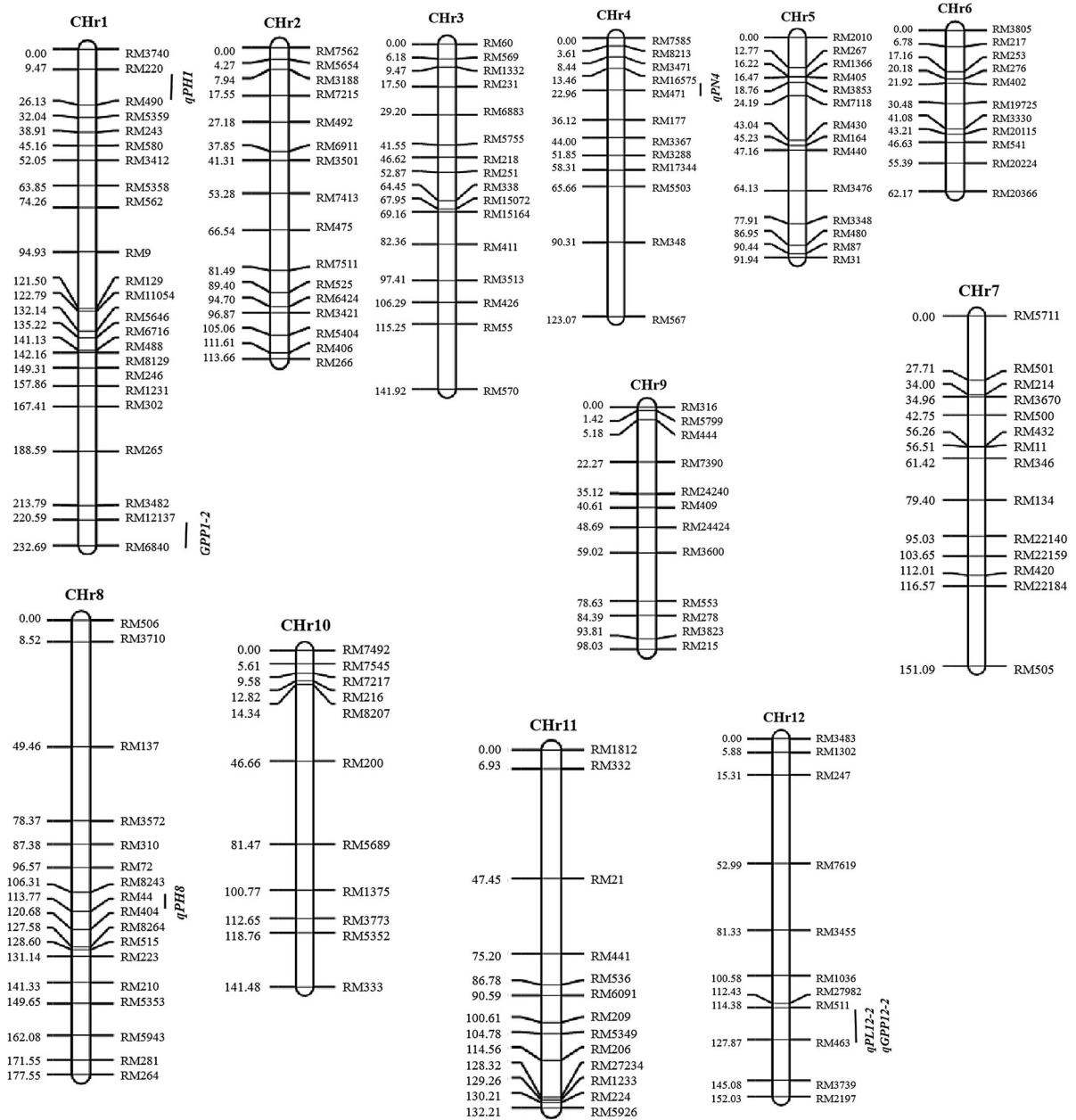
Fig. 4 Interval distribution of QTLs for cold tolerance-related traits in the IAPAR-9/Liaoyan241 RIL population. *PH* plant height, *PL* panicle length, *PN* number of panicles per plant, *GPP* grain number per panicle

QTLs and some related to cold tolerance. There were 2 QTLs, *qPL12-2* and *qGPP12-2*, which correlated with cold resistance and were located in the adjacent region, RM511–RM463, in the RIL of IAPAR-9/ Liaoyan241. So, there was an important area for cold tolerance. Compared with previous studies, this area has been mapped to many QTLs related to cold

resistance. These QTLs were detected both at vegetative and reproductive stages (Fig. 5). Among them, *qCTS12-1*, a QTL related to cold tolerance at the seedling stage was fine-mapped in 77 kb of RM28506–R1709 on chromosome 12 (Liu et al. 2009). Han et al. (2005) detected a QTL *qCTB12* that was related to cold-tolerance at the booting stage by
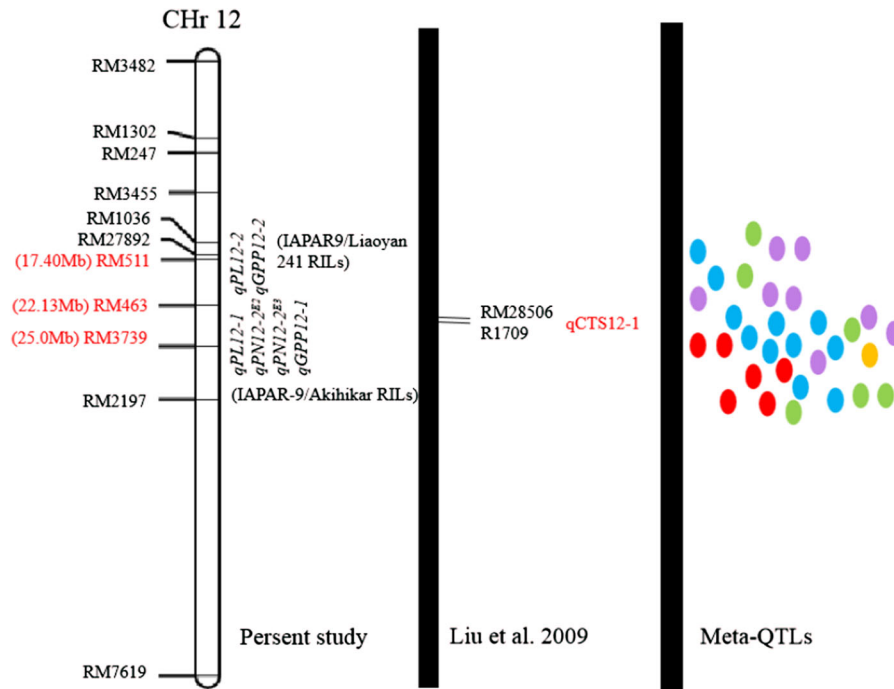
Fig. 5 The meta-analysis of some cold tolerance QTLs on chromosome 12 in previously studies. Red circles: booting stage (Han et al. 2005; Zeng et al. 2009; Shinada et al. 2013; Yang 2016; Shakiba et al. 2017). Blue circles: seedling stage (Liu et al. 2009; Schläppi et al. 2017; Luo et al. 2018; Han et al. 2007; Mao et al. 2015; Jian 2011; Sun 2015; Lv et al. 2016; Zhang et al. 2018). Orange circles: tillering stage (Zhang and Zou 2012). Green circles: bud stage (Shakiba et al. 2017; Luo et al. 2016). Violet circles: germination stage (Zhang et al. 2018; Ji et al. 2008; Hou et al. 2004; Sales et al. 2017). (Color figure online)

using a hybrid population from Jileng 1 and Miyang 23. Shinada et al. (2013) detected 3 QTLs related to cold-tolerance at the booting stage by using a hybrid population from Eikei88223 and Suisei, in which $qCTF12$ located near RM463–RM3739. Yang (2016) mapped several cold tolerance QTLs at the seedling and booting stages by using Single Segment Substitution Lines (SSSL). Schläppi et al. (2017) also detected 2 QTLs associated with cold resistance in the seeding stage, $qLTSS12-1$ and $qLTSS12-2$. Shakiba et al. (2017) detected 4 QTLs associated with cold resistance in the germination stage and 1 QTL associated with cold resistance in the booting stage by GWAS. Luo et al. (2016) detected 2 QTLs, $qCTS-12-3$ and $qCTS-12-4$, related to cold tolerance in the bud stage of rice. Therefore, the RM511–RM3739 region on chromosome 12 is related to cold tolerance in rice. In this experiment, multiple QTLs related to cold tolerance were detected in this region, and further study will be required to identify the specific genes and their locations within the interval.

Conclusion

In order to locate QTLs related to agronomic traits and cold temperature stress in rice, two RIL populations were constructed using cold-sensitive rice IAPAR-9 and cold-tolerant rice Akihikari and Liaoyan241. The two RIL populations and the parental lines were planted under both natural and cold water irrigation stress condition in Gongzhuling, Jilin and under natural low temperature stress condition in Kunming, Yunnan. For each, agronomic traits were identified at the maturity stage. Phenotypic identification showed that low temperature had a strong influence on the number of panicles per plant and seed setting rate; QTL results showed that $qPN12-2^{E2}$ and $qPN12-2^{E3}$ were detected in the IAPAR-9/Akihikari population grown under cold water irrigation stress in Gongzhuling, Jilin and under natural low temperature conditions in Kunming, Yunnan. These QTLs were stable across two different environments. Based on our findings from two RIL populations, the RM511–RM3739

region on chromosome 12 is likely an important region for rice cold-tolerance.

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