Euphytica (2016) 208:251–264
DOI 10.1007/s10681-015-1584-z

Genetic analysis of germinating ability and seedling vigor under cold stress in US weedy rice

Anna H. Borjas · Teresa B. De Leon · Prasanta K. Subudhi

Received: 6 March 2015 / Accepted: 21 October 2015 / Published online: 27 October 2015

Abstract Low temperature stress is a major constraint for rice production in temperate and high altitude areas of the world. Delayed germination coupled with reduced seedling vigor hinders crop establishment and crop growth resulting in reduced rice productivity. Mapping of the chromosomal regions controlling cold tolerance will accelerate marker-assisted breeding of cold tolerant rice varieties. A recombinant inbred line mapping population involving a US weedy rice accession 'PSRR-1' and a rice cultivar 'Bengal' was evaluated for germinating ability and seedling vigor under low temperature (13 °C) and optimum temperature (28 °C). 'PSRR-1' performed better than 'Bengal' under cold stress. Forty-nine QTL distributed over ten chromosomes were identified for 11 traits. The number of QTL varied from one to nine with phenotypic variability of each QTL ranging from 3.5 to 12.7 %. For 18 QTL, 'Bengal' alleles were desirable, whereas 'PSRR-1' allele improved germination and seedling vigor under cold stress in 31 QTL. Three major QTL were observed for coleoptile length and seedling shoot length. The QTL were clustered in six chromosomal regions. The congruency of QTL cluster on chromosome 11 with earlier studies suggests a potential target for cloning cold tolerance genes at germination and seedling stages. This study demonstrated that weedy rice can be a valuable donor for desirable alleles to improve germination and seedling stage cold tolerance in rice.

Keywords Cold tolerance · Germination · *Oryza sativa* · RIL population · Seedling vigor · Weedy rice

A. H. Borjas · T. B. De Leon · P. K. Subudhi (✉)
School of Plant, Environmental, and Soil Sciences, Louisiana State University Agricultural Center, Baton Rouge, LA 70803, USA
e-mail: psubudhi@agcenter.lsu.edu

Introduction

Rice is cultivated all over the world with a wide range of variation in geography. Compared with the tropical and subtropical regions, rice grown in temperate and high altitude regions is frequently exposed to low temperature during the growing season. Cold stress is a major constraint to rice cultivation in 25 countries, including the United States (Yoshida 1981; Pereira da Cruz et al. 2013). Although rice is sensitive to cold stress, wide range of genetic variation for cold tolerance exists in rice germplasm (Baruah et al. 2009; Bosetti et al. 2012) and *indica* cultivars are less tolerant than the *japonica* cultivars (Andaya and Mackill 2003).

In direct seeded rice, germination ability, early seedling emergence, and seedling vigor are crucial for rapid and uniform crop establishment, and increased ability to compete with weeds in rainfed and upland rice cropping system. Staggered germination results in

variation in maturity resulting in rice crop with poor quality. Fast germination and seedling growth under low temperature is a major breeding objective particularly in direct seeding cropping system in rice growing areas experiencing low temperature stress.

Rice plants are damaged by exposure to cold stress during all stages of plant growth. The optimal temperature range for germination and seedling establishment is 25–35 °C (Chapman and Peterson 1962). However, a number of factors such as varietal difference, duration of cold stress, and physiological stage of the plant are responsible for variation in cold tolerance of rice (Yoshida 1981; Nishiyama 1985). In rice breeding, cold tolerance is usually evaluated at germination, seedling, and reproductive stages. Since field evaluation is challenging, methodologies to evaluate cold tolerance in controlled condition have been developed. Jones and Peterson (1976) developed a slant board test procedure to evaluate seedling vigor under cold stress and noted that shoot length of 15-d old seedling at 18 °C is significantly correlated with seedling vigor in field condition. Correlation between coleoptile length and seedling establishment under low temperature in field condition has been reported (Ogiwara and Terashima 2001). The evaluation of germination index, seedling survival percentage, coleoptile length, radicle length, seedling length, and root length under low temperature has been a common practice in a wide range of studies such as germplasm and cold tolerant near-isogenic lines characterization (Cruz and Milach 2004; Sharifi 2010; Zhou et al. 2012), inheritance studies (Sthapit and Witcombe 1998), and QTL mapping (Fujino et al. 2004).

A number of QTL mapping studies focused on germination under cold stress (Miura et al. 2001; Fujino et al. 2004; Chen et al. 2006; Ranawake et al. 2014) and early seedling cold tolerance (Zhang et al. 2005a; Lou et al. 2007; Liu et al. 2015). In Nipponbare × Kasalath cross, Miura et al. (2001) identified five QTL for germination under low temperature stress on chromosomes 2, 4, 5, and 11 with a total phenotypic variance of 41 %. Nipponbare alleles were beneficial in case of two QTL while Kasalath improved germination under cold stress in rest. Chen et al. (2006) used an *indica* × *japonica* cross and identified two QTL for low temperature germination ability on chromosomes 3 and 10. Fujino et al. (2004) identified three QTL on chromosomes 3 and 4 for low temperature germination ability in a cross involving temperate *japonica* varieties. Of these, the major QTL *qLTG3-1* encoding a protein of unknown function was cloned (Fujino et al. 2008).

Ranawake et al. (2014) studied cold tolerance at germination (CTG) and early seedling (CTS) stages in a *japonica* × *indica* cross. The cold tolerance QTL in their study were dependent on growth stages. Using seedling survival percentage after cold stress as a criterion, multiple QTL were mapped in several studies (Zhang et al. 2005a; Lou et al. 2007; Liu et al. 2015). In another study, Zhang et al. (2005b) identified 34 QTL for four seedling vigor traits (germination rate, root length, shoot length, and dry weight) at three temperature regimes (25, 20, and 15 °C). Most of these were concentrated in five chromosomal regions with individual QTL contribution ranging from 3 to 16 % of the phenotypic variance. Significant genotype × temperature regime interaction was observed. Among the four seedling vigor traits, shoot length and germination rate were concluded as good predictor for seedling vigor in rice. Combining QTL mapping with micro-array data, Liu et al. (2013) reported a candidate gene (LOC_Os07g22494) that improved cold tolerance at early seedling stage.

Only one QTL mapping study used a recombinant inbred line (RIL) population involving a japonica weedy rice, which was evaluated for cold tolerance at the reproductive stage (Oh et al. 2004). This study demonstrated the utility of weedy rice as potential donor in rice breeding. Although weedy rice belongs to the same genus and species as cultivated rice (Hoagland and Paul 1978), it is noted for its persistence and survival due to its unusual genetic variability and phenotypic plasticity (Oka 1988). The weedy rice infestation is a major challenge for the rice farmers in southern United States and many parts of the world (Webster 2000). Molecular marker-based investigation showed that weedy rices are closer to *indica* or *japonica* subspecies of rice rather than the wild species *Oryza rufipogon* (Suh et al. 1997). But weedy rice accessions of the United States were genetically more diverse and some accessions were found to be closer to *O. nivara* or *O. rufipogon* (Vaughan et al. 2001). Weedy rice has many desirable attributes such as rapid seedling growth, higher root mass, deeper root, seedling resistance to pathogen attack (Lee et al. 2000) and tolerance to various biotic and abiotic stresses (Suh et al. 1997, 1999), which can be

exploited to boost rice productivity. Unlike wild species of rice, weedy rice hybridizes easily making fertile hybrids and facilitates development of populations for investigating the genetics of useful agronomic and domestication traits.

In this study, we demonstrated that the US weedy rice has enhanced seedling vigor even under cold stress and further identified QTL for cold tolerance and seedling vigor based on seedling attributes in a mapping population involving a US weedy rice accession. Our results suggest that US weedy rice can be an invaluable resource for rice breeding programs to improve both germination and seedling vigor under cold stress.

Materials and methods

Plant materials

A population of 198 RILs in the $F_{7:8}$ generation was developed from the cross between a medium grain high yielding rice cultivar 'Bengal' (Linscombe et al. 1993) and a weedy rice accession PSRR-1 (Subudhi et al. 2012). PSRR-1 was collected from the Rice Research Station at Crowley, LA and was purified by single plant selection for two generations before crossing to develop the mapping population. It has light green leaves, vigorous growth, long auricles and ligules, straw-hulled medium grains, lax open panicles, and pubescent leaves. This weedy rice accession is extremely susceptible to shattering and has a higher intensity of both hull and pericarp dormancy compared to Bengal.

Evaluation of cold tolerance

For each RIL and parents, seeds were treated at 50 °C for 5 days to eliminate residual dormancy. Before initiating the experiment, eight randomly selected RILs and parents were tested for germination. After the germination was tested, seeds of the RIL population and parents were placed in petri dishes lined with a layer of germinating paper (Anchor Paper Co.) with addition of 10 mL of distilled water. The petri dishes were then placed in a basin covered with Saran wrap and transferred to an incubator set at 28 °C for pregermination in darkness. After 2 days of incubation at 28 °C, the pregerminated seeds were transferred to incubators for germination in dark condition under optimum temperature (28 °C) and cold stress (13 °C) for 7 and 14 days, respectively.

A randomized block design with three replications was followed for cold tolerance evaluation. For each line, 20 seeds were used for germination in each replication. Besides germination percentage, observations were recorded on five randomly selected germinating seeds for coleoptile length (CL) and radicle length (RL). For normal condition, the measurements were taken only on the 7th day after incubation. But for cold stress, the measurements were recorded on the 7th and 14th day after incubation. Splitting of the hull by the emerging radicle was used as the criterion for visible germination. The number of seeds germinated in each replication of each RIL and parent was expressed as a percentage. The percent germination was arcsine transformed to improve the normality of distribution for statistical analysis and QTL mapping.

To evaluate the response of parents and individual RIL, cold response index (CRI) was calculated using the formula [CRI = (Phenotypic value after 7 days of incubation at 13 °C)/(Phenotypic value after 7 days of incubation at 28 °C) × 100]. The degree of susceptibility to cold stress at germination stage was ascertained from the CRI values.

All traits were abbreviated as follows: germination percent after 7 days of incubation at 28 °C (Germ7d28C), 13 °C (Germ7d13C), and 14 days of incubation at 13 °C (Germ14d13C); coleoptile length after 7 days of incubation at 28 °C (CL7d28C), 13 °C (CL7d13C), and 14 days of incubation at 13 °C (CL14d13C); radicle length after 7 days of incubation at 28 °C (RL7d28C), 13 °C (RL7d13C) and 14 days of incubation at 13 °C (RL14d13C); CRI for germination (CRI-Germ), CRI for coleoptile length (CRI-CL), CRI for radicle length (CRI-RL).

After evaluating cold tolerance in the laboratory, the germinated seeds were transferred to the greenhouse with 26/18 °C day/night temperature in order to assess the development of the plants in soil. The experiment was conducted in a randomized block design with three replicates. Ten germinated seeds per each RIL were sown in a tray in each replication and five random plants per line were measured for shoot length (StL-GH) and root length (RtL-GH) at14 days after planting in the greenhouse.

Statistical analyses

Analysis of variance (ANOVA) and mean comparison were done using the GLIMMIX procedure. Lines were entered as the fixed effect and the replications were treated as random effect. To improve the normality, data were transformed prior to analysis. Percent germination data were arcsine transformed while the coleoptile and radicle length data were transformed by taking the square root of the value anchored to 0.5. Pearson correlation coefficients among traits were computed based on RIL means using the CORR procedure and broad-sense heritability of each trait was estimated following Holland et al. (2003). All analyses were carried out using SAS (SAS Institute 2011). All histograms were made in Microsoft Excel 2010.

QTL analysis

A linkage map developed in this RIL population (Subudhi et al. 2012) was used for QTL mapping. The linkage map consisted of 212 simple sequence repeat (SSR) markers and a morphological marker *Rc* (the pericarp color) with a total map distance of 1410 cM and average marker interval of 6.6 cM. Both single marker analysis and composite interval mapping (CIM) were performed using QTL Cartographer version 2.5 (Wang et al. 2011). In the CIM procedure, a forward–backward regression procedure with 20 cofactors was followed with walk in speed of 1.0 cM for detection of QTL. Logarithm of odds (LOD) score of 2.5 was used as the threshold for declaring significance of the QTL. Since QTL identified using data taken after 14 days of exposure at 13 °C were same as those from 7 days at 13 °C, those data were not provided. The QTL were named following McCouch et al. (1997). For example, the QTL located on chromosome 3 for germination percentage after 7 days of exposure to 28 °C was named as *qGerm7d28C-3*.

Results

Variation in early seedling vigor and cold tolerance response among parents and RILs

Analysis of variance revealed significant differences with respect to all traits in the RIL population except RL7d28C. Descriptive statistics with respect to germination and early seedling vigor traits under both normal and cold stress were presented in Table 1. Bengal and PSRR-1 did not differ in the germination rate 7 days after incubation at 28 °C, but there were significant differences in coleoptile length and radicle length (Fig. 1). For both traits, weedy rice accession PSRR-1 showed better performance compared with Bengal.

Low temperature stress reduced germination percentage significantly in 'Bengal' after 7 and 14 days. However, PSRR-1 maintained the same germination rate under cold stress like the optimum condition. At both time points, germination remained around 70 % in Bengal. Cold stress seriously damaged the coleoptile and radicle growth. But PSRR-1 grew significantly faster compared with Bengal. To compare the performance of both parents under cold stress relative to optimum condition, cold response index was calculated. For all three traits, PSRR-1 was better than Bengal. To observe the impact of cold stress at early seedling stage, seedlings were planted in the greenhouse under normal temperature for 14 days. PSRR-1 had significantly greater shoot length compared to Bengal, whereas there was no difference in root length.

There was a wide range of variability in the population. For the traits, Germ7d28C, Germ7d13C, and Germ14d13C, distribution was skewed (Fig. 2). The distribution for coleoptile and radicle length at both optimum and low temperatures was normal (Fig. 3). The distribution for CRI (CL) was normal but the CRI for other two traits was a little skewed (Fig. 4). The traits, StL-GH and RtL-GH, were normally distributed with many of the RILs falling outside of the parental range indicating transgressive segregation (Fig. 5). Mean values of the RIL population were lower than both parents for Germ7d28C, Germ7d13C, CL7d28C, and RL7d28C but higher than the parent with higher mean for CL7d13C, CRI (CL), and CRI (RL) and similar or within the parental range in the rest.

Broad sense heritability was moderate to high for Germ7d28C, Germ7d13C, Germ14d13C, CL7d28C, CRI (Germ), CRI (CL), and RL7d13C, and was low for CL7d13C, RL7d28C, CRI (RL), StL-GH, and RtL-GH.

Table 1 Descriptive statistics for cold tolerance-related traits during germination and early seedling stage in Bengal × PSRR-1 RIL mapping population of rice

Trait	Bengal mean	PSRR-1 mean[a]	BR-RILs mean	BR-RILs range	Pr > F[b]	Heritability
Germ7d28C	99.99	99.99[ns]	75.55 ± 0.86	19.50–89.36	<0.0001	0.92
Germ7d13C	86.67	99.99**	59.81 ± 1.40	4.73–89.36	<0.0001	0.67
Germ14d13C	88.33	99.99**	70.28 ± 1.12	4.73–89.36	<0.0001	0.87
CL7d28C (mm)	48.33	73.00**	39.83 ± 0.64	4.20–70.20	<0.0001	0.80
CL7d13C (mm)	0.47	2.87*	3.02 ± 0.10	0.07–7.40	<0.0001	0.20
RL7d28C (mm)	71.33	92.00[ns]	43.62 ± 0.91	3.20–78.13	0.0014	0.38
RL7d13C (mm)	0.60	4.40*	2.96 ± 0.13	0.00–10.53	<0.0001	0.17
CRI (Germ)	77.06	100.00**	77.74 ± 1.49	9.62–126.68	<0.0001	0.79
CRI (CL)	0.97	3.93[ns]	7.54 ± 0.25	0.69–22.42	<0.0001	0.80
CRI (RL)	0.84	4.78*	7.24 ± 0.39	0.02–30.99	0.0105	0.79
StL-GH (cm)	27.93	31.33*	23.04 ± 0.39	4.80–34.67	<0.0001	0.40
RtL-GH (cm)	6.47	5.31[ns]	7.79 ± 0.13	2.20–12.07	<0.0001	0.29

Germ7d28C, arcsine transformed values of germination% after 7 days at 28 °C; Germ7d13C, arcsine transformed values of germination percentage after 7 days at 13 °C; Germ14d13C, arcsine transformed values of germination% after 14 days at 13 °C; CL7d28C, coleoptile length after 7 days at 28 °C; CL7d13C, coleoptile length after 7 days at 13 °C; RL7d28C, radicle length after 7 days at 28 °C; RL7d13C, radicle length after 7 days at 13 °C; CRI-Germ, cold response index for germination; CRI-CL, cold response index for coleoptile length; CRI-RL, cold response index for radicle length; StL-GH, shoot length in greenhouse experiment; RtL-GH, root length in greenhouse experiment

ns not significant

* Significant at $P < 0.05$, ** significant at $P < 0.01$

[a] Differences in trait means between Bengal and PSRR-1

[b] Significant difference among the RILs of the Bengal × PSRR-1 mapping population based on *F* test from the ANOVA

Correlations among traits

As shown in Table 2, the phenotypic correlations among all germination and early vigor traits were all positive and highly significant ($P < 0.001$). The only negative and significant correlation was observed between CRI-RL and RL7d28C. The germination percentage under optimum temperature was highly correlated to traits measured under both optimum and low temperature stress, but the radicle length under optimum condition or root length under greenhouse was either not correlated with many of these traits or the strength of most correlations was low. The cold response index for coleoptile length and radicle length did not show correlation with the seedling traits measured in greenhouse after exposure to cold stress.

Quantitative trait loci for germination ability and seedling growth under cold stress

Scanning of the whole genome detected 49 QTL for eleven traits distributed along ten chromosomes under both control and low temperature stress environments (Table 3; Fig. 6). Thirty QTL were clustered within six regions of five chromosomes 1, 3, 8, 11, and 12. The number of QTL varied between one and nine per trait and each QTL contributed 3.5–12.7 % of the total phenotypic variation. The weedy PSRR-1 alleles increased the trait means in 31 QTL and Bengal alleles were favorable in the rest.

Further examination of QTL results revealed the following points. Only one QTL was detected for Germ7d28C with Bengal allele improving germination percentage, but three QTL were detected under cold stress including the one for Germ7d28C. For the two QTL, PSRR-1 allele improved germination under cold stress. For the cold response index, two of the four QTL were same as the QTL for Germ7d13C and two were new.

For traits, CL7d28C, CL7d13C, and CRI-CL, 6, 8, and 9 QTL were detected, respectively (Table 3; Fig. 6). Only one QTL on chromosome 2 was common under both optimum and cold temperature. Five QTL located on chromosomes 1, 3, 8, and 12 were identical

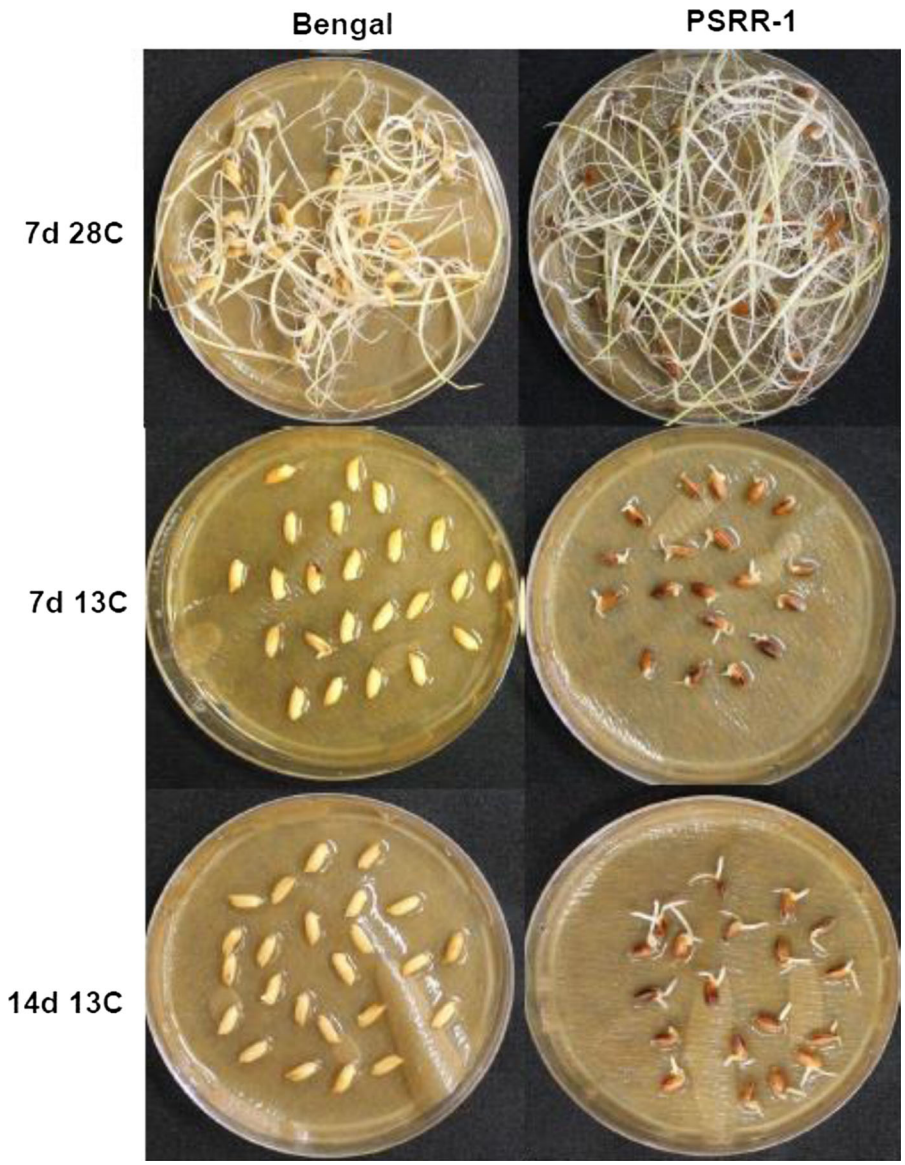


Fig. 1 Germination of parents under optimum temperature and cold stress. Pictures were taken 7 days after incubation at 28 °C (*top*) and 13 °C (*middle*), and 14 days after incubation at 13 °C (*bottom*)

with respect to the direction of additive effect between CRI-CL and CL7d13C. Only one major QTL from PSRR-1 accounting for a phenotypic variation of 11 % was located on chromosome 8. For RL7d28C and RL7d13C, three QTL were detected for each trait, but six QTL were identified for CRI-RL. In case of two of the 3 QTL for CL7d28C, Bengal allele was desirable. There were two common QTL on identical position for these traits. None of these QTL explained phenotypic variation greater than 10 %. There were three QTL each identified for StL-GH and RtL-GH and PSRR-1 contributed the allele for cold tolerance in case of two QTL. There was no overlapping of QTL between them. But there was one major QTL on chromosome 11 explaining 11 % of phenotypic variation and desirable allele was from PSRR-1.

Based on the radicle length and coleoptile length under normal condition of growth, PSRR-1 showed improved seedling vigor compared with Bengal. But the QTL mapping results for both traits under

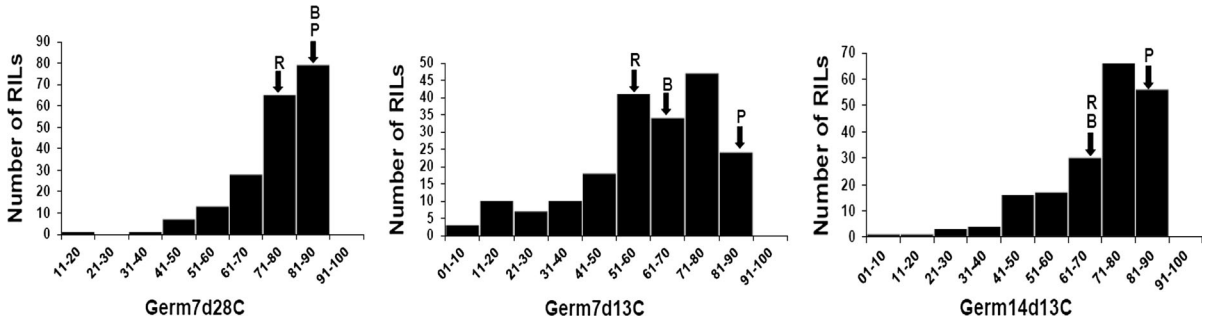


Fig. 2 Frequency distribution of germination% under cold stress in the RIL population of the cross Bengal × PSRR-1. Mean values for parents and RIL population are marked by *arrows*. *B*, *P*, and *R* represent for Bengal, PSRR-1, and RIL population, respectively. Germ7d28C, Arcsine transformed values of germination% after 7 days at 28 °C; Germ7d13C, Arcsine transformed values of germination% after 7 days at 13 °C; Germ14d13C, Arcsine transformed values of germination% after 14 days at 13 °C

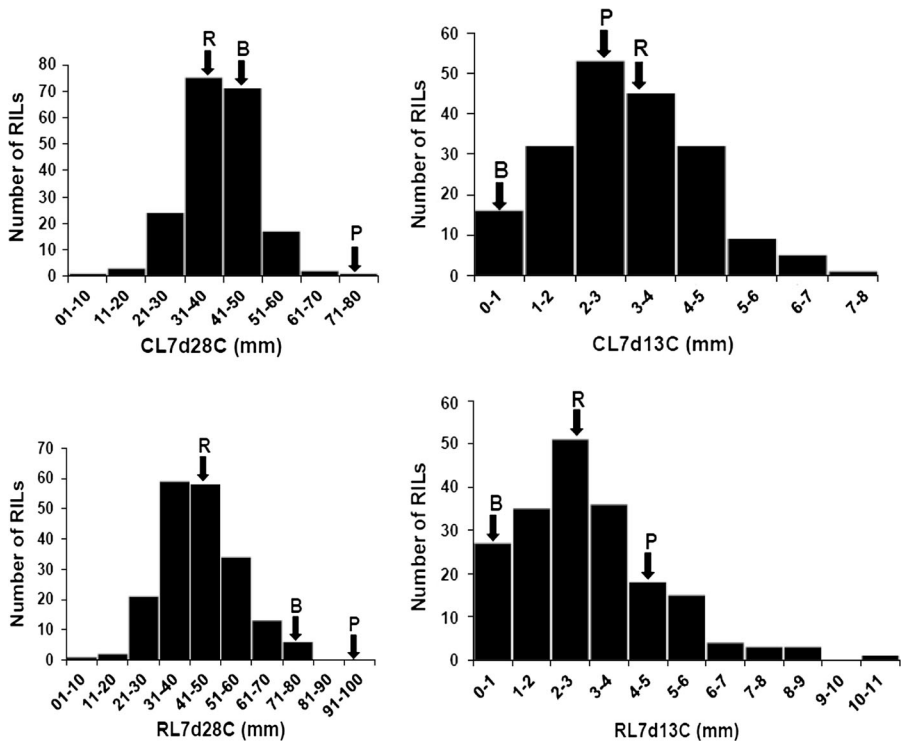


Fig. 3 Frequency distribution of coleoptile length and radicle length under cold stress in the RIL population of the cross Bengal × PSRR-1. Parental means are marked by *arrows*. *B*, *P*, and *R* represent mean values for Bengal, PSRR-1, and RIL population, respectively. CL7d28C, Coleoptile length after 7 days at 28 °C; RL7d28C, Radicle length after 7 days at 28 °C; CL7d13C, Coleoptile length after 7 days at 13 °C; RL7d13C, Radicle length after 7 days at 13 °C; CL14d13C, Coleoptile length after 14 days at 13 °C; RL14d13C, Radicle length after 14 days at 13 °C

optimum environments revealed that both parents had genes or QTL, which can improve seedling vigor. For example, there were two QTL each for CL7d28C and RL7d28C with Bengal allele providing the alleles with positive effects. Other traits measured under cold stress followed a similar trend with both parents contributing alleles to improve germination ability and early seedling vigor under low temperature stress.

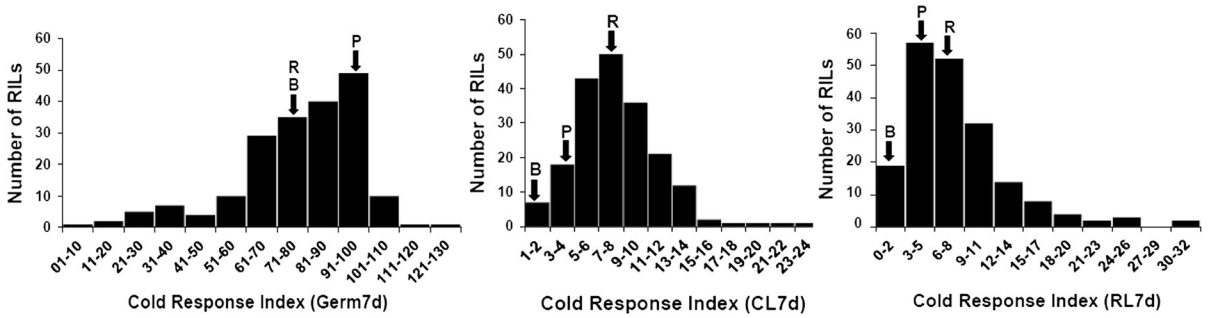


Fig. 4 Frequency distribution of cold response indices in RIL population of the cross Bengal × PSRR-1. Mean values for parents and RIL population are marked by *arrows*. *B*, *P*, and *R* represent for Bengal, PSRR-1, and RIL population, respectively. Cold response indices were calculated as [(trait mean at 13 °C/trait mean at 28 °C) × 100]

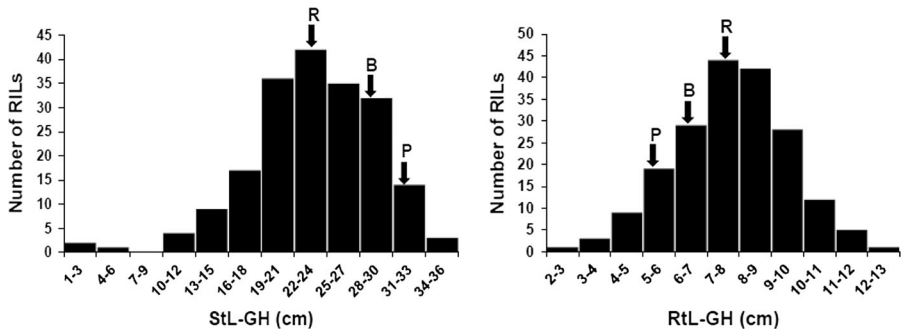


Fig. 5 Frequency distribution of shoot length and root length 14 days after transplanting in greenhouse in RIL population of the cross Bengal × PSRR-1. The germinated seeds were transferred to greenhouse after the cold tolerance evaluation in the laboratory. Mean values for parents and RIL population are marked by *arrows*. *B*, *P*, and *R* represent for Bengal, PSRR-1, and RIL population, respectively. StL-GH, shoot length in greenhouse experiment; RtL-GH, root length in greenhouse experiment

Discussion

Low temperature in temperate and high altitude areas of the tropical regions is a major hindrance to rice cultivation. It has a detrimental effect on germination, seedling growth, and reproductive success by interfering with the metabolism (Zhang et al. 2014a, b). Since tolerance to low temperature at a particular stage of crop growth is not correlated to cold tolerance at other developmental stages (Kaw and Khush 1985; Zhou et al. 2012), QTL studies had been conducted to investigate cold tolerance at various growth and developmental stages such as germination stage (Miura et al. 2001; Fujino et al. 2004), seedling stage (Kim et al. 2014; Zhang et al. 2014a, b; Liu et al. 2015), vegetative stage (Andaya and Mackill 2002), and booting stage (Andaya and Mackill 2003; Oh et al. 2004). In this study, we focused on germination ability and early seedling growth under cold stress, which are important factors for uniform stand establishment in rice growing areas of southern USA, where cold temperature prevents germination in dry seeded rice.

There was no difference in germination rate under optimum temperature. Therefore, it was obvious that we detected only one QTL from the cultivated rice suggesting little genetic difference between both parents with respect to germination ability at optimum temperature. However, there was a strong contrast between both parents for germination ability under cold stress, which was supported by detection of four QTL on chromosomes 1, 7, 11, and 12 controlling this trait and PSRR-1 alleles in all cases were superior to Bengal (Table 3). The reduction in coleoptile length and radicle length was higher compared to observation in Japanese rice germplasm (Bosetti et al. 2012).

Table 2 Pearson correlation coefficients between the early seedling stage cold tolerance and seedling vigor traits in the RIL mapping population of rice cross Bengal × PSRR-1

	Germ7d28C	Germ7d13C	CL7d28C	RL7d28C	CL7d13C	RL7d13C	CRI-Germ	CRI-CL	CRI-RL	StL-GH	RtL-GH
Germ7d28C	1.000										
Germ7d13C	0.739***	1.000									
CL7d28C	0.565***	0.602***	1.000								
RL7d28C	0.396***	0.325***	0.390***	1.000							
CL7d13C	0.450***	0.715***	0.469***	0.157*	1.000						
RL7d13C	0.426***	0.645***	0.341***	0.019	0.750***	1.000					
CRI-Germ	0.436***	0.912***	0.506***	0.225**	0.697***	0.605***	1.000				
CRI-CL	0.288***	0.548***	0.058	0.009	0.883***	0.669***	0.581***	1.000			
CRI-RL	0.289***	0.491***	0.177*	−0.298***	0.637***	0.907***	0.489***	0.648***	1.000		
StL-GH	0.351***	0.510***	0.387***	0.323***	0.299***	0.218**	0.479***	0.179*	0.089	1.000	
RtL-GH	0.243***	0.281***	0.198**	0.157*	0.141	0.150*	0.219**	0.073	0.099	0.338***	1.000

Significance of correlation coefficients were indicated by * (P values < 0.05), ** (P values < 0.01), and *** (P values < 0.001)

Since PSRR-1 was superior to Bengal with respect to the coleoptile length and radicle length under optimal temperature regime (Table 1), it provided an opportunity to identify the genomic regions responsible for such attributes related to seedling vigor. There was no colocalisation of the QTL for coleoptile length and radicle length indicating different genes responsible for these traits. The QTL alleles from both parents contributed to the improvement of seedling vigor under optimum temperature.

Comparison of QTL positions on the rice genome with those detected in prior studies provided information about the QTL consistency despite the differences in marker types, the genetic materials, and screening methodology. Miura et al. (2001) reported five QTL on chromosomes 2, 4, 5, and 11 controlling germination ability under cold stress. Three of these QTL on chromosomes 2, 5, and 11 were located in similar positions in our study. Fujino et al. (2004) identified 3 QTL on chromosomes 3 and 4 with one major QTL *qLTG-3-1* explaining 35 % of phenotypic variation, which was later cloned (Fujino et al. 2008). However, we did not detect any QTL on corresponding locations of both chromosomes. Baruah et al. (2009) reported five QTL for cold tolerance at the plumule and seedling stage in a cross between a *japonica* rice A58 and an wild rice accession W107 (*O. rufipogon*). The QTL on chromosome 11, which was consistent at plumule and seedling stages, also overlapped with the same genomic region harboring several QTL for cold tolerance attributes in our study (Fig. 6). Few other studies reported 2-5 QTL or genomic regions controlling these attributes (Chen et al. 2006; Zhang et al. 2005b; Ranawake et al. 2014). There was no overlapping of QTL detected in our study with those identified by Chen et al. (2006). Three QTL on chromosomes 6 and 7 for germination ability under cold stress and two QTL on chromosomes 6 and 11 for cold tolerance at seedling stage (Ranawake et al. 2014) coincided with our study. Several studies reported QTL for cold tolerance traits such as seedling vigor (Zhang et al. 2005a; Baruah et al. 2009), tolerance to seedling stage necrosis (Andaya and Mackill 2003), and seedling stage cold tolerance (Kim et al. 2014) in the similar region on chromosome 11. Considering all these reports, it is clear that this chromosome 11 location might be a hot spot for genes responsible for cold tolerance at germination and seedling stages. A candidate gene, ORF LOC_Os11g37720 (*Duf6* gene),

Table 3 Chromosome location, additive effects, direction of phenotypic effect, and estimate phenotypic variance of the putative QTL associated with early seedling stage cold tolerance and seedling vigor detected in the RIL population of the cross Bengal × PSRR-1 using composite interval mapping procedure

Trait[a]	QTL	Peak marker	Position[b]	LOD value	Additive effect[c]	R^{2d}	DPE[e]
Germ7d28C	*qGerm728-3*	RM15499	96.4	4.1	3.465	7.6	B
Germ7d13C	*qGerm713-3*	RM16	92.2	3.683	4.673	5.1	B
	qGerm713-7	RM125	36.7	3.156	−4.746	4.9	P
	qGerm713-11	RM254	96.7	6.902	−6.860	9.5	P
CRI-Germ	*qCRI-Germ-1*	RM312	41.7	3.901	−6.417	5.4	P
	qCRI-Germ-7	Rc	44.4	2.526	−4.981	3.7	P
	qCRI-Germ-11	RM206	95.5	6.529	−8.283	9.6	P
	qCRI-Germ-12	RM101	39.7	2.631	−4.746	3.9	P
CL7d28C	*qCL728-2*	RM5512	38.3	2.640	−1.881	4.0	P
	qCL728-6	RM7193	58.3	2.580	1.795	3.7	B
	qCL728-7-1	RM6574	36.0	4.317	−2.581	6.8	P
	qCL728-7-2	RM5508	71.4	3.992	2.550	5.7	B
	qCL728-7-3	RM429	90.4	2.504	−1.941	3.5	P
	qCL728-11	RM1341	83.7	2.806	−2.066	4.8	P
CL7d13C	*qCL713-1-1*	RM220	4.4	3.538	−0.434	5.0	P
	qCL713-1-2	RM580	41.0	3.039	−0.337	4.1	P
	qCL713-2	RM5512	34.3	2.781	−0.347	5.1	P
	qCL713-3	RM16	93.2	6.751	0.473	9.7	B
	qCL713-8	RM331	43.5	5.600	−0.611	11.2	P
	qCL713-11	RM254	95.7	3.775	−0.344	5.0	P
	qCL713-12-1	RM101	39.7	5.979	−0.427	8.3	P
	qCL713-12-2	RM28661	88.5	6.101	0.478	9.4	B
CRI-CL	*qCRI-CL-1*	RM283	4.7	6.047	−1.106	8.3	P
	qCRI-CL-2	RM262	76.7	3.477	0.919	5.6	B
	qCRI-CL-3	RM15499	94.4	3.228	0.755	4.2	B
	qCRI-CL-5	RM159	0.0	3.176	0.747	4.1	B
	qCRI-CL-8	RM331	40.5	2.692	−0.776	3.6	P
	qCRI-CL-10	RM1146	69.5	4.016	−0.886	5.6	P
	qCRI-CL-11	RM26092	26.5	4.012	0.917	6.3	B
	qCRI-CL-12-1	RM101	39.7	4.808	−0.919	6.5	P
	qCRI-CL-12-2	RM28661	86.5	3.270	0.875	5.0	B
RL7d28C	*qRL728-1*	RM11706	117.2	5.726	−4.073	9.6	P
	qRL728-8	RM72	31.8	6.060	4.671	8.9	B
	qRL728-12	RM5479	80.8	3.137	3.017	5.0	B
RL7d13C	*qRL713-1*	RM312	41.7	3.003	−0.434	5.1	P
	qRL713-12-1	RM101	39.7	2.836	−0.427	4.9	P
	qRL713-12-2	RM28661	87.5	3.873	0.543	7.2	B
CRI-RL	*qCRI-RL-1-1*	RM312	41.7	5.124	−1.497	6.8	P
	qCRI-RL-1-2	RM466	56.8	3.764	−1.395	5.4	P
	qCRI-RL-3	RM15499	94.4	4.073	1.329	5.4	B
	qCRI-RL-6	RM4924	56.5	2.781	1.201	3.8	B
	qCRI-RL-8	RM331	41.5	4.724	−1.591	7.2	P
	qCRI-RL-12	RM7619	39.4	4.697	−1.419	6.5	P

Table 3 continued

Trait[a]	QTL	Peak marker	Position[b]	LOD value	Additive effect[c]	R^{2d}	DPE[e]
StL-GH	*qStL-GH-1*	RM8278	130.9	6.834	−2.098	12.7	P
	qStL-GH-3	RM16	93.2	5.233	1.655	7.9	B
	qSL-GH-11	RM254	98.7	6.044	−1.951	11.1	P
RtL-GH	*qRtL-GH-1*	RM580	41.0	3.421	0.491	5.5	B
	qRtL-GH-6	RM190	3.7	2.878	−0.403	4.5	P
	qRtL-GH-10	RM6100	57.4	4.584	−0.529	7.9	P

[a] Germ7d28C, Arcsine transformed values of germination% after 7 days at 28 °C; Germ7d13C, Arcsine transformed values of germination% after 7 days at 13 °C; CRI-Germ, cold response index for germination; CL7d28C, Coleoptile length after 7 days at 28 °C; CL7d13C, Coleoptile length after 7 days at 13 °C; CRI-CL, cold response index for coleoptile length; RL7d28C, Radicle length after 7 days at 28 °C; RL7d13C, Radicle length after 7 days at 13 °C; CRI-RL, cold response index for radicle length; StL-GH, shoot length in greenhouse experiment; RtL-GH, root length in greenhouse experiment

[b] QTL peak position on the linkage map

[c] Additive effects of Bengal allele

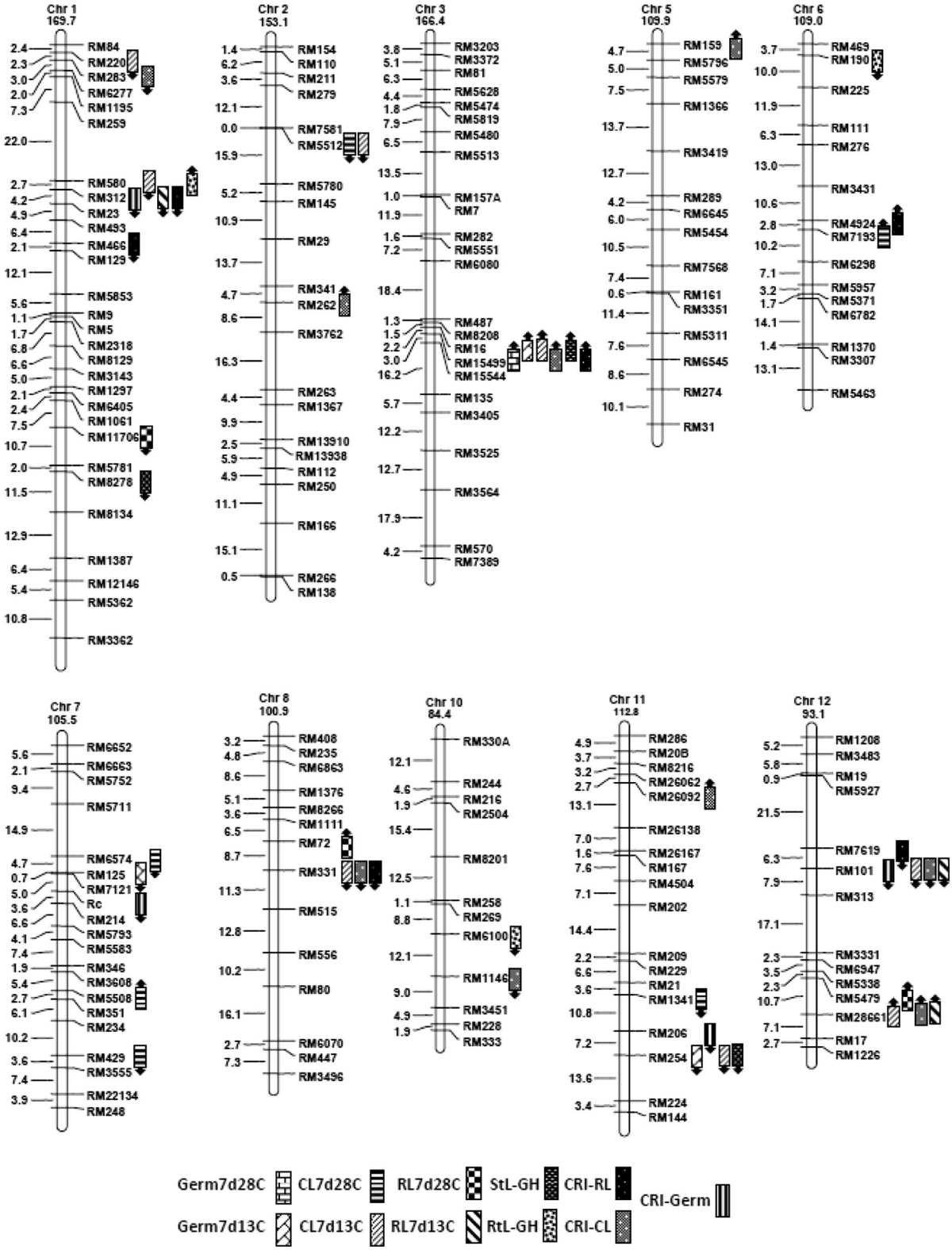
[d] Phenotypic variation (%) explained by each QTL

[e] DPE, direction of phenotypic effect. B and P denote Bengal and PSRR-1 alleles increasing the phenotypic values, respectively

co-segregating with seedling cold tolerance was reported (Kim et al. 2014). Further exploration of this region using the introgression line PSRR-1 (Subudhi et al. 2015) should lead to cloning of the genes responsible for various component traits of cold tolerance.

Mapping of seedling vigor, which was assessed in both laboratory and greenhouse condition revealed that genes controlling seedling vigor under cold stress were different from those under normal temperature (Fig. 6). When QTL results for all traits were considered in totality, we observed that there were six clusters of QTL for multiple seedling vigor and germination traits under cold stress on chromosomes 1, 3, 8, 11, and 12. For the two clusters on chromosomes 3 and 12, positive alleles for all cold tolerance traits were from 'Bengal', whereas weedy rice alleles improved cold tolerance in the remaining 4 clusters. Strong correlations observed among the traits (Table 2) were consistent with QTL mapping results because the majority of QTL for both germination ability and seedling vigor traits were colocalized in few chromosomal regions with the same parental allele either decreasing or increasing the trait mean (Fig. 6; Table 3). It is interesting to note that most of the congruent QTL were not localized in the QTL clusters detected in this study with the exception of that on chromosome 11. It appears that these QTL are novel compared with the earlier reports (Miura et al. 2001; Fujino et al. 2004; Zhang et al. 2005b; Chen et al. 2006; Ranawake et al. 2014). Most of these studies used *indica* × *japonica* cross with the exception of Fujino et al. (2004), who used the population developed from the cross between two *japonica* varieties. Unlike the above studies, the mapping population used in our study involved a *japonica* variety and a weedy rice accession. Therefore the discrepancy in QTL detection could be due to the differences in the genetic background of the plant materials. Zhang et al. (2005b) reported five genomic regions where QTL for multiple traits were evaluated to assess seedling vigor. Each cluster was designated as a putative QTL, which could be either cluster of linked loci or single locus with pleiotropic effects. Another finding was that two StL-GH QTL and one RtL-GH QTL were placed in these clusters along with other seedling and germination attributes, which suggest that these traits could be used as reliable indicators to evaluate seedling vigor under cold stress (Zhang et al. 2005b).

The weedy rice accession PSRR-1 was collected from the rice field in Louisiana, USA, where cold stress is common during the rice cropping season. High level of seed germination ability under cold stress in this weedy rice accession might be due to strong selection pressure for cold tolerance and early seedling vigor (Mackill and Lei 1997; Baruah et al. 2009). Weedy rice populations from different


Chr 1
169.7
RM84
RM220
RM283
RM6277
RM1195
RM259
RM580
RM312
RM23
RM493
RM466
RM129
RM5853
RM9
RM5
RM2318
RM8129
RM3143
RM1297
RM6405
RM1061
RM11706
RM5781
RM8278
RM8134
RM1387
RM12146
RM5362
RM3362
Chr 2
153.1
RM154
RM110
RM211
RM279
RM7581
RM5512
RM5780
RM145
RM29
RM341
RM262
RM3762
RM263
RM1367
RM13910
RM13938
RM112
RM250
RM166
RM266
RM138
Chr 3
166.4
RM3203
RM3372
RM81
RM5628
RM5474
RM5819
RM5480
RM5513
RM157A
RM7
RM282
RM5551
RM6080
RM487
RM8208
RM16
RM15499
RM15544
RM135
RM3405
RM3525
RM3564
RM570
RM7389
Chr 5
109.9
RM159
RM5796
RM5579
RM1366
RM3419
RM289
RM6645
RM5454
RM7568
RM161
RM3351
RM5311
RM6545
RM274
RM31
Chr 6
109.0
RM469
RM190
RM225
RM111
RM276
RM3431
RM4924
RM7193
RM6298
RM5957
RM5371
RM6782
RM1370
RM3307
RM5463
Chr 7
105.5
RM6652
RM6663
RM5752
RM5711
RM6574
RM125
RM7121
Rc
RM214
RM5793
RM5583
RM346
RM3608
RM5508
RM351
RM234
RM429
RM3555
RM22134
RM248
Chr 8
100.9
RM408
RM235
RM6863
RM1376
RM8266
RM1111
RM72
RM331
RM515
RM556
RM80
RM6070
RM447
RM3496
Chr 10
84.4
RM330A
RM244
RM216
RM2504
RM8201
RM258
RM269
RM6100
RM1146
RM3451
RM228
RM333
Chr 11
112.8
RM286
RM208
RM8216
RM26062
RM26092
RM26138
RM26167
RM167
RM4504
RM202
RM209
RM229
RM21
RM1341
RM206
RM254
RM224
RM144
Chr 12
93.1
RM1208
RM3483
RM19
RM5927
RM7619
RM101
RM313
RM3331
RM6947
RM5338
RM5479
RM28661
RM17
RM1226
Germ7d28C
CL7d28C
RL7d28C
StL-GH
CRI-RL
CRI-Germ
Germ7d13C
CL7d13C
RL7d13C
RtL-GH
CRI-CL

◀**Fig. 6** Chromosomal location of QTL for traits related to early seedling stage cold tolerance and seedling vigor in the RIL population developed from the cross Bengal × PSRR-1 (Subudhi et al. 2012). QTL are shown on the *left side* of each chromosome. *Bars* with *upward pointing arrows* indicate increasing effect on trait mean by Bengal allele and *bars* with *downward pointing arrows* indicate PSRR allele increasing the trait mean. Germ7d28C, Arcsine transformed values of germination% after 7 days at 28 °C; Germ7d13C, Arcsine transformed values of germination% after 7 days at 13 °C; CRI-Germ, cold response index for germination; CL7d28C, Coleoptile length after 7 days at 28 °C; CL7d13C, Coleoptile length after 7 days at 13 °C; CRI-CL, cold response index for coleoptile length; RL7d28C, Radicle length after 7 days at 28 °C; RL7d13C, Radicle length after 7 days at 13 °C; CRI-RL, cold response index for radicle length; StL-GH, shoot length in greenhouse experiment; RtL-GH, root length in greenhouse experiment

geographic location of China have been reported to evolve rapidly with respect to their germination response due to genetic differentiation (Chen et al. 2004; Xia et al. 2011). Despite many weedy attributes, weedy rice could be an important resource for genetic improvement of crop plants like other wild relatives (Lu and Ellstrand 2014). We showed that the four QTL clusters on chromosomes 1, 8, 11, and 12, where weedy rice alleles were beneficial, would be useful for marker-assisted selection to introduce a high level of cold tolerance and seedling vigor to rice cultivars grown in the USA. Since we observed transgressive variation for both improved germination and vigor under cold stress in the mapping population, it is likely that these QTL alleles from weedy rice in combination with the favorable alleles of the cultivated rice would result in rice varieties with improved cold tolerance. Consequently, cloning of these QTL using the introgression lines of this weedy rice accession developed in a cultivated background (Subudhi et al. 2015) should aid in the precise transfer of the cold tolerance attributes from the weed rice.

Funding This research was funded by United States Department of Agriculture-National Institute of Food and Agriculture (Grant Number 2006-35320-16555). This manuscript was approved for publication by the Director of Louisiana Agricultural Experiment Station, USA as manuscript number 2015-306-22571.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Andaya VC, Mackill DJ (2002) QTLs conferring cold tolerance at the booting stage of rice using recombinant inbred lines from japonica x indica cross. Theor Appl Genet 106:1084–1090

Andaya VC, Mackill DJ (2003) Mapping of QTLs associated with cold tolerance during the vegetative stage in rice. J Exp Bot 54:2579–2585

Baruah AR, Ishigo-Oka N, Adachi M, Oguma Y, Tokizono Y, Onishi K, Sano Y (2009) Cold tolerance at the early growth stage in wild and cultivated rice. Euphytica 165:459–470

Bosetti F, Montebelli C, Novembre ADLC, Chamma HP, Pinheiro JB (2012) Genetic variation of germination cold tolerance in Japanese rice germplasm. Breed Sci 62:209–215

Chapman AL, Peterson ML (1962) The seedling establishment of rice under water in relating to temperature and dissolved oxygen. Crop Sci 2:391–395

Chen H, Xuan S, Wang W, Shao G, Sun Z (2004) Freezing tolerance and germination ability at low temperature of Dandong weedy rice. Chin J Rice Sci 18:109–112

Chen L, Lou Q, Sun Z, Xing Y, Yu X, Luo L (2006) QTL mapping of low temperature on germination rate of rice. Rice Sci 13:93–98

Cruz RP, Milach SCK (2004) Cold tolerance at the germination stage of rice: methods of evaluation and characterization of genotypes. Sci Agric 61:1–8

Fujino K, Sekiguchi H, Sato T, Kiuchi H, Nonoue Y, Takeuchi Y, Ando T, Lin SY, Yano M (2004) Mapping of quantitative trait loci controlling low-temperature germinability in rice (*Oryza sativa* L.). Theor Appl Genet 108:794–799

Fujino K, Sekiguchi H, Matsuda Y, Sugimoto K, Ono K, Yano M (2008) Molecular identification of a major quantitative trait locus, *qLTG3-1*, controlling low-temperature germinability in rice. Proc Natl Acad Sci USA 105:12623–12628

Hoagland RE, Paul RN (1978) A comparative SEM study of red rice and several commercial rice (*Oryza sativa*) varieties. Weed Sci 26:619–625

Holland JB, Nyquist WE, Cervantes-Martinez CT (2003) Estimating and interpreting heritability for plant breeding: an update. Plant Breed Rev 22:9–111

Jones DB, Peterson ML (1976) Rice seedling vigor at suboptimal temperatures. Crop Sci 16:102–105

Kaw R, Khush G (1985) Combining ability for low-temperature tolerance in rice. Rice Genet I:593–612

Kim SM, Suh JP, Lee CK, Lee JH, Kim YG, Jena KK (2014) QTL mapping and development of candidate gene-derived DNA markers associated with seedling cold tolerance in rice (*Oryza sativa* L.). Mol Genet Genomics 289:333–343

Lee FN, Gealy DR, Holiday RH (2000) Differential response of United States *Oryza sativa* (red rice) accessions to races of *Pyricularia grisea*. In: Beyrouty CA (ed) BR Wells Rice Research Studies-1999, Series 476. Arkansas Agricultural Experiment Station, Arkansas, pp 94–101

Linscombe SD, Jodari F, Mckenzie KS, Bollich PK, White LM, Groth DE, Dunand RT (1993) Registration of Bengal rice. Crop Sci 33:645–646

Liu F, Xu W, Song Q, Tan L, Liu J, Zhu Z, Fu Y, Su Z, Sun C (2013) Microarray-assisted fine mapping of quantitative trait loci for cold tolerance in rice. Mol Plant 6:757–767

Liu W, Lu T, Li Y, Pan X, Duan Y, Min J, Fu X, Sheng X, Xiao J, Liu S, Tan J, Yao Y, Li X (2015) Mapping of quantitative trait loci for cold tolerance at the early seedling stage in landrace rice Xiang 743. Euphytica 201:401–409

Lou Q, Chen L, Sun Z, Xing Y, Li J, Xu X, Mei H, Luo L (2007) A major QTL associated with cold tolerance at seedling stage in rice (*Oryza sativa* L.). Euphytica 158:87–94

Lu B-R, Ellstrand NC (2014) World food security and the tribe Triticeae (Poaceae): genetic resources of cultivated, wild, and weedy taxa for crop improvement. J Syst Evol 52:661–666

Mackill DJ, Lei X (1997) Genetic variation for traits related to temperate adaptation of rice cultivars. Crop Sci 37:1340–1346

McCouch SR, Cho YG, Yano M, Paul E, Blinstrub M (1997) Report on QTL nomenclature. Rice Genet Newsl 14:11–13

Miura K, Lin SY, Yano M, Nagamine T (2001) Mapping quantitative trait loci controlling low-temperature germinability in rice (*Oryza sativa* L.). Breed Sci 51:293–299

Nishiyama I (1985) Physiology of cool weather damage to rice plant. Hokkaido University Press, Sapporo, p 325

Ogiwara H, Terashima K (2001) A varietal difference in coleoptile growth is correlated with seedling establishment of direct seeded rice in submerged field under low-temperature conditions. Plant Prod Sci 4:166–172

Oh CS, Choi YH, Lee SJ, Yoon DB, Moon HP, Ahn SN (2004) Mapping of quantitative trait loci for cold tolerance in weedy rice. Breed Sci 54:373–380

Oka HI (1988) Weedy forms of rice. In: Oka MI (ed) Origin of cultivated rice. Elsevier and Japanese Sci Soc Press, Amsterdam Tokyo, pp 107–114

Pereira da Cruz R, Sperotto RA, Cargnelutti D, Adamski JM, de FreitasTerra T, Fett JP (2013) Avoiding damage and achieving cold tolerance in rice plants. Food Energy Secur 2:96–119

Ranawake AL, Manangkil OE, Yoshida S, Ishii T, Mori N, Nakamura C (2014) Mapping QTLs for cold tolerance at germination and the early seedling stage in rice (*Oryza sativa* L.). Biotechnol Biotechnol Equip 28:989–998

SAS Institute Inc (2011) SAS® 9.3 system options: reference, 2nd edn. SAS Institute Inc, Cary

Sharifi P (2010) Evaluation on sixty-eight rice germplasms in cold tolerance at germination stage. Rice Sci 17:77–81

Sthapit BR, Witcombe JR (1998) Inheritance of tolerance to chilling stress in rice during germination and plumule greening. Crop Sci 38:660–665

Subudhi PK, De Leon T, Singh PK, Parco A, Cohn MA, Sasaki T (2015) A chromosome segment substitution library of weedy rice for genetic dissection of complex agronomic and domestication traits. PLoS ONE 10(6):e0130650

Subudhi PK, Parco A, Singh PK, DeLeon T, Karan R, Biradar H, Cohn MA, Brar DS, Sasaki T (2012) Genetic architecture of seed dormancy in us weedy rice in different genetic backgrounds. Crop Sci 52:2564–2575

Suh HS, Sato Y, Morishima H (1997) Genetic characterization of weedy rice (*Oryza sativa* L.) based on morpho-physiology, isozymes, and RAPD markers. Theor Appl Genet 94:316–321

Suh JP, Ahn SN, Moon HP, Suh HS (1999) QTL analysis of low temperature germinability in weedy rice. Korean J Breed 31:261–267

Vaughan LK, Ottis BV, Prazak-Havey AM, Sneller C, Chandler JM, Park WD (2001) Is all red rice found in commercial rice really *Oryza sativa*? Weed Sci 49:468–476

Wang S, Basten CJ, Zeng ZB (2011) Windows QTL Cartographer 2.5. Department of Statistics, North Carolina State University, Raleigh, NC. http://statgen.ncsu.edu/qtlcart/WQTLCart.htm. Accessed 29 Dec 2014

Webster TM (2000) Weed survey-southern states: grass crops subsection. Proc South Weed Sci Soc 53:247–274

Xia HB, Xia H, Ellstrand NC, Yang C, Lu BR (2011) Rapid evolutionary divergence and ecotypic diversification of germination behavior in weedy rice populations. New Phytol 191:1119–1127

Yoshida S (1981) Fundamentals of rice crop science. Growth and development of the rice plant, vol 1. IRRI, cap, Los Banos, pp 1–63

Zhang ZH, Qu XS, Wan S, Chen LH, Zhu YG (2005a) Comparison of QTL controlling seedling vigor under different temperature conditions using recombinant inbred lines in rice (*Oryza sativa*). Ann Bot 95:423–429

Zhang ZH, Su L, Wei L, Chen W, Zhu YG (2005b) A major QTL conferring cold tolerance at the early seedling stage using recombinant inbred lines of rice (*Oryza sativa* L.). Plant Sci 168:527–534

Zhang Q, Chen Q, Wang S, Hong Y, Wang Z (2014a) Rice and cold stress: methods for its evaluation and summary of cold tolerance-related quantitative trait loci. Rice 7:24

Zhang S, Zheng J, Liu B, Peng S, Leung H, Zhao J, Wang X, Yang T, Huang Z (2014b) Identification of QTLs for cold tolerance at seedling stage in rice (*Oryza sativa* L.) using two distinct methods of cold treatment. Euphytica 195:95–104

Zhou L, Zeng Y, Hu G, Pan Y, Yang S, You A, Zhang H, Li J, Li Z (2012) Characterization and identification of cold tolerant near-isogenic lines in rice. Breed Sci 62:196–201