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MAPPING QUANTITATIVE TRAIT LOCI FOR COLD TOLERANCE IN RICE AT SEEDLING STAGE

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Abstract

Rice plants are sensitive to cold stress at various growth stages starting from germination to maturity. Exploitation of cold tolerance for the development of cold tolerant varieties may help in saving crop loss or cost of production in the cold prone environment. Introgression of quantitative trait loci (QTLs) conferring cold tolerance in the elite variety may be the most effective and sustainable approach to address this issue. A $F_{2:3}$ mapping population of a cross between a cold susceptible variety (BR1) and a cold tolerant variety (BR18) was used to map QTLs for cold tolerance at seedling stage. Three significant QTLs on chromosomes 1, 3 and 12 associated with cold tolerance were mapped between the markers intervals of RM220-RM10829, RM546-RM7 and RM27877-RM463, respectively at a threshold LOD of 4.3 through composite interval mapping. Phenotypic contributions of these QTLs were 50.7, 39 and 52.7%, respectively. Marker information of such a large effect QTL could be used in the breeding program after necessary validation to develop cold tolerant high yielding rice varieties.

Introduction

Cold stress is one of the major abiotic stresses affecting rice crop at seedling stage in the northern parts of Bangladesh. Germination and seedling establishment are very sensitive growth stages of rice to cold stress. Seedling mortality ranging from 10 - 90% is observed in some years at early establishement stage of Boro rice crop in the northern districts of Bangladesh when severe cold spell occurs in late December to mid January. This entails increased cost of production for replanting, which is a great problem for the marginal farmers. Farmers sometimes delay planting to avoid seedling mortality caused by low temperature, which eventually lowers the grain yield of Boro rice due to exposure of reproductive phase in high temperature in April. To overcome this problem, use of rice varieties tolerant to cold stress at seedling stage could be a sustainable crop management approach. In the cold prone area in Bangladesh, farmers commonly grow major high yielding varieties like, BRRI dhan28 and BRRI dhan29 but they lack cold tolerance. Although, there are other few cold tolerant rice varieties (BRRI dhan36 and BRRI dhan55) that were developed through conventional breeding method by BRRI, but their performance is not good enough to address the cold injury problem in Boro rice. Furthermore, the rice varieties which are popularly grown in South and South-east Asian countries and are preferred by the consumers usually lack cold tolerance. However, there are rice germplasm that can tolerate cold stress and can maintain better growth than the intolerant one under low temperature stress conditions. Exploitation and use of cold tolerance from such germplasm in development of high yielding rice varieties could save crop loss or cost of cultivation. Therefore, exploring genetic resources to develop cold tolerant rice variety is essential. Molecular analysis of QTLs underlying cold tolerance is the best approach to develop cold tolerant varieties.

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Several reports on quantitative trait loci (QTL) for seedling stage cold tolerance in rice is available in literature (Andaya and Mackill 2003, Lou *et al.* 2007, Zhi-Juan *et al.* 2010, Kim *et al.* 2012, Suh *et al.* 2012, Yang *et al.* 2013, Park *et al.* 2013). Marker information and corresponding donor germplasm of these studies can be used to develop cold tolerant and high yielding rice varieties through marker assisted selection. However, the donor germplasms used in these studies are of *japonica* origin. The crosses between *indica* and *japonica* rice usually produce progenies with higher spikelet sterility due to inter-subspecific reproductive barrier. Furthermore, *japonica* types are not adaptable to tropical condition and are not preferred by the consumers in South Asian countries like Bangladesh due to their sticky cooked rice. Use of *indica* donors in the breeding program to develop cold tolerant rice is the best way to avoid such unexpected linkage drag in the high yielding background, although level of cold tolerance in *indica* rice is poor and occurrence of higher tolerance is extremely rare. BRRI has identified BR18 as a potential donor for cold tolerance at seedling stage (Biswas *et al.* 2012). Therefore, this study was undertaken to map QTLs from a $F_{2:3}$ mapping population of BR1 × BR18.

Materials and Methods

A set of 856 $F_{2:3}$ mapping population derived from a cross between BR1 and BR18 was used for this study. All the field and laboratory work for this study were conducted at Plant Breeding Division of Bangladesh Rice Research Institute, Gazipur, Bangladesh in collaboration with Department of Botany, Jahangirnagar University, Savar, Dhaka, Bangladesh. BR1 is a cold susceptible high yielding variety at seedling stage. On the other hand, BR18 is cold tolerant rice variety at seedling stage. Although BR18 is a high yielding variety developed by BRRI for Haor regions of the country, its tall stature does not fit to the cold prone northern districts of Bangladesh.

The $F_{2:3}$ progenies were evaluated for cold tolerance using cold treatment of constant 13°C in a cold-water tank following Khatun *et al.* (2016). Briefly, seeds were sown in plastic trays filled with gravels and crop residue free granular soil with 3 replicates. Ten seedlings of each entry were raised at ambient temperature at each replication. At three-leaf stage (12 - 14 days after seeding), the plastic trays were placed in cold water tanks preset at 13°C. The depth of water in the tank was maintained at 5 cm. Temperature of cold water tank was kept at constant 13°C for 6 days or until the susceptible check variety dies. An arbitrary leaf discoloration (LD) score~~s~~ (1 to 9; 1 means all leaves are green and 9 means seedlings are almost dead) was assigned to each entry considering magnitude of discoloration of leaves due to low temperature treatment on the day of LD scoring following standard evaluation system (SES) of IRRI (IRRI 2013).

Parental polymorphism information of around 560 SSR markers distributed over rice genome for BR1 and BR18 was analyzed using method described by Syed *et al*. (2016). Briefly, DNA was extracted from young and actively growing fresh leaves using miniprep modified CTAB method. Polymerase chain reaction (PCR) was performed in 10 µl volume containing 2 µl of genomic DNA, 5.3 µl of DDH_2O, 1 µl of 1X PCR buffer, 1 µl of 0.1mM dNTP mix, 0.5 µl of 0.25 µM of each primer, 2 µl of Taq polymerase of 1U. The temperature cycles were programmed at 94°C for 5 min (initial denaturation), 94°C for 30 sec (denaturation), 55°C for 30 sec (primer annealing), 72°C for 60 sec (extension), 72°C for 5 min (final extension) and 10°C forever (storage). The PCR product was separated on 6% polyacrylamide gel through electrophoresis for 1.5 to 2.5 hrs and was visualized in a gel documentation system. Parental polymorphism was determined based on the comparative deviation in band position.

Selective genotyping technique (Lander and Botstein 1989) was used to select plants for genotyping. A set of 40 F_2 plants along with their parents were used in genotyping with 76

polymorphic SSR markers distributed over 12 chromosomes of rice. Allele scoring was performed based on comparative position of the parental DNA bands. The progeny having band at same level of BR18's band was scored as 'A' and the progeny having band at same level of BR1's band was scored as 'B' while heterozygous alleles were scored as 'H' (Fig.1). .

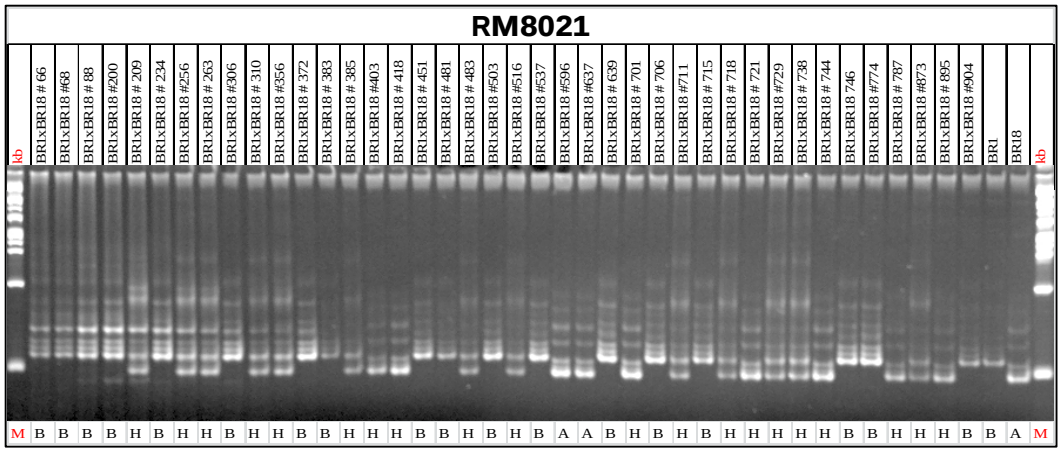

Fig. 1. A partial gel image of genotyping of 40 F_2 progenies along with parental lines with RM8021; M represents marker of 1 Kb DNA ladder; A, B and H represent homozygous BR18, homozygous BR1 allele, heterozygous allele, respectively.

Construction of linkage map and detection of QTLs for cold tolerance were performed using QGene4.0 (Nelson 1997). Each marker was analysed through single marker analysis (SMA). To be more precise about the location of the identified QTLs, interval mapping (IM) and composite interval mapping (CIM) analysis were performed. Permutation test was performed with 1000 interations to declar threshold LOD (Logarithm of Odds) for significant QTL at 5% level of probability. The proportion of the total phenotypic variation explained by each QTL was calculated as R^2 value (R^2 = Ratio of the sum of squares explained by the QTL to the total sum of squares). The QTLs were named based on the nomenclature suggested by McCouch *et al*. (1997).

Results and Discussion

Cold tolerance at seedling stage of 856 $F_{2:3}$ progenies was evaluated using cold induced leaf discoloration (LD) pattern at low temperature (13ºC). Analysis of variance (ANOVA) showed that the progenies had significantly variable LD values ranging from 1 to 9 with an average of 5.1 (Table 1). The LD values showed a moderate heritability estimate and apparently normal frequency distribution pattern (Fig. 2) having skewness and kurtosis very close to unity. On the other hand, the susceptible parent BR1 showed significantly higher LD value than that of BR18. Continuous variation in frequency distribution in cold induced leaf discoloration and cold induced necrosis was also reported by Andaya and Mackill (2003), in percent seedling survival by Lou *et al.* (2007) and Zhang *et al.* (2005), in seedling height and seedling dry weight by Han *et al.* (2007) and germination rate by Ranawake *et al.* (2014). Continuous variation in frequency distribution reveals that the trait is quantitatively inherited, which warrants QTL mapping. The skewness and kurtosis values of the frequency distribution that were very close to unity also supported the suitability of the phenotypic data for QTL analysis.

Table 1. Descriptive statistics of leaf discoloration scores of 856 $F_{2:3}$ progenies.

Descriptive statistics	Value
Parents	
LD value of BR1	8.8** ± 0.12
LD value of BR18	2.1 ± 0.19
$F_{2:3}$ progenies	
Average	5.1
Range	1 - 9
CV	30.16
P- value	0.0002
Skew	0.09
Kurtosis	–0.78
h^2b	40.4

LD, leaf discoloration, **, significant at 1 % level of probability.

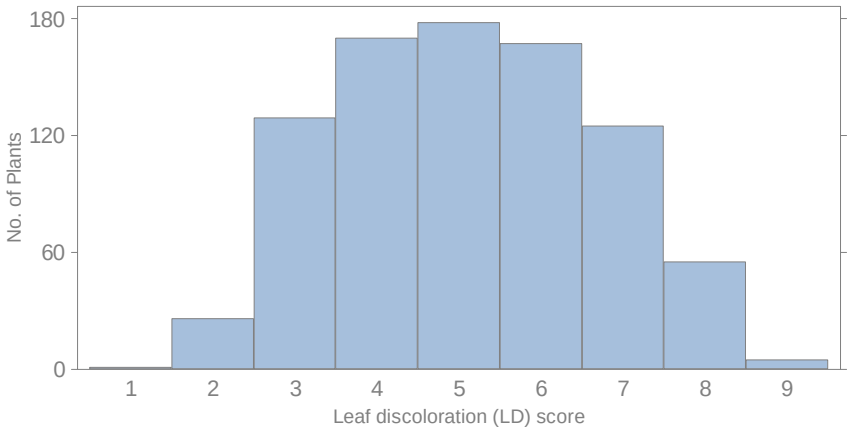

Fig. 2. Frequency distribution of LD scores in 856 $F_{2:3}$ families of BR1 × BR18.

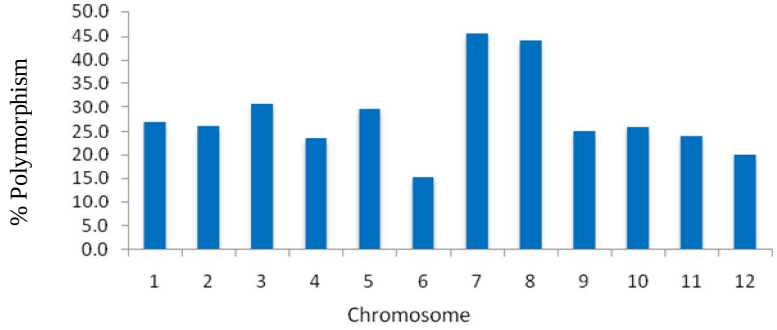

Fig. 3. Chromosome-wise SSR marker polymorphism between BR1 and BR18.

A parental survey was conducted with 560 microsatellite markers to identify at least one polymorphic marker per 20 cM throughout the genome of the parental lines. Fig. 3 shows the percentage of polymorphic SSRs distributed on rice chromosomes. The polymorphism rate across 12 chromosomes ranged from 15.2 to 45.7% with an average of 28%. The highest polymorphism rate was observed on chromosome 7 followed by on chromosome 8 (43.9%) and chromosome 3 (30.6%). The minimum polymorphism rate was observed on chromosome 6 (15.2%). The extent of polymorphism between the parental lines depends on the genetic distance between them. The closely related cultivars (*indica* × *indica* or *japonica* × *japonica*) usually show lower polymorphism than the genetically diverged cultivars (*indica* × *japonica*). The lower polymorphism rate in present study might be due to their *indica* origin. Septiningsih *et al*. (2012) reported 115 polymorphic and reliable SSR markers out of 1,074 (10.5%). Similar results were obtained when linkage map was constructed using a *japonica / japonica* mapping population (Bing *et al*. 2006).

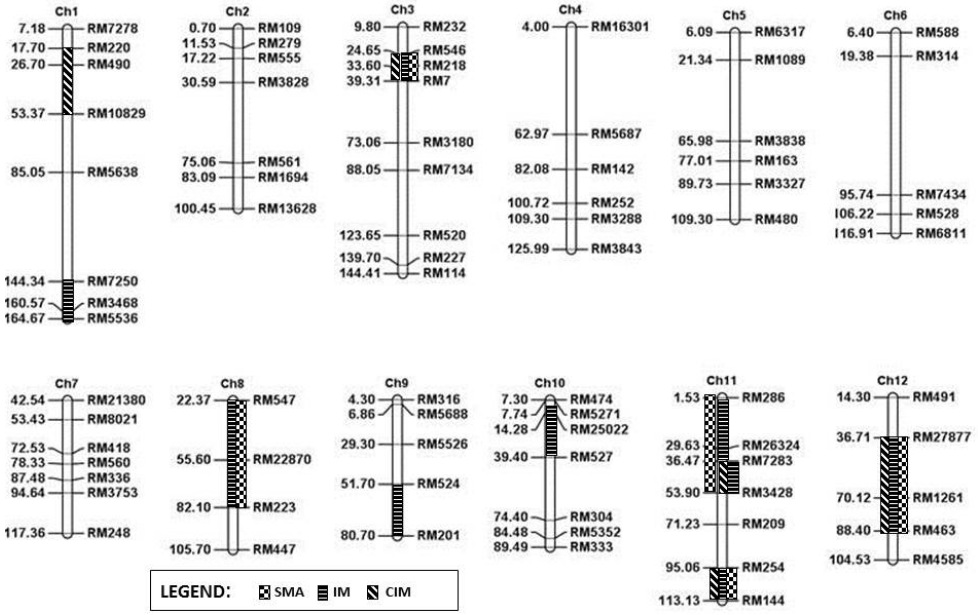

Fig. 4. Linkage map of 40 $F_{2:3}$ families from BR1× BR18 with 76 polymorphic SSR markers using QGene 4.0. QTLs detected through SMA, IM and CIM were overlaid on respective chromosomal position.

Linkage map of 12 chromosomes was constructed with 76 polymorphic SSR markers using QGene 4.0 (Nelson 1997) (Fig. 4). The map covered 1346.1 cM of rice genome in length (cM data was obtained by multiplying corresponding physical distance with 3.96) with an average marker interval of 19.5 cM. There were still a few gaps larger than 20 cM on all the chromosomes. The progenies from the extreme tails of frquency distribution of LD score were considered for genotyping following selective genotyping technique (Lander and Botstein 1989). This genotyping technique not only helps truncate many F_2 population into a judicious sized mapping population with no significant sacrifice of phenotypic variation but also it enriches frequencies of contrasting alleles and thus it is a commonly used genotyping strategy to reduce genotyping cost (Collard *et al*. 2005). In the present study, out of 856 $F_{2:3}$ progenies, 40 plants that account for around 2.3% progenies from either upper and bottom tails of the frequency distribution were sampled for

genotyping (Fig. 5). The truncated chunk of the genotypes had contrasting LD values of 2.6 - 4.2 at bottom tail and 7.8 - 9.0 at upper tail, which were closer to the parental LD values (Table 1).

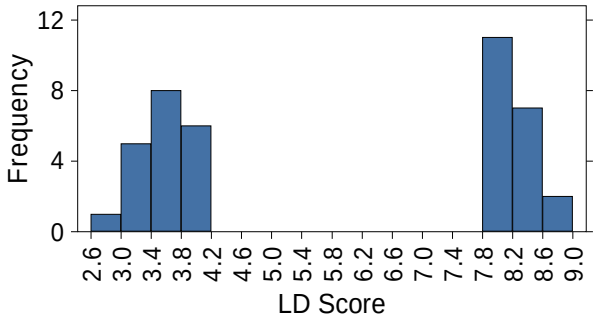

Fig. 5. Frequency distribution of LD score of the selcted progenies from the extreme ends of cold sensitivity.

Genotyping of 40 F_2 plants with 76 polymorphic SSR markers covering 1346.1cM of rice genome reveals 11 putative chromosomal segments responsible for cold tolerance when genotype and phenotype data were subjected to SMA, SIM and CIM analysis (Fig. 4). Out of 11 QTLs, six, eight and five QTLs were identified through single marker analysis, interval mapping and composite interval mapping, respectively. Three QTLs were common among three methods of QTL detection. Maximum four QTLs were mapped on chromosome 11 and single QTL was mapped on each of chromosomes 3, 8, 9 and 12. Two QTLs were detected on chromosome 1. Out of five QTLs that were detected through CIM, three QTLs were found significant at a threshold LOD of 4.3 at 5% level of significance in permutation test with 1000 iterations (Table 2).

Table 2. Putative QTLs identified for seedling stage cold tolerance from $F_{2:3}$ mapping population of BR1×BR18 through single marker analysis, interval mapping and composite interval mapping.

[a] QTL name	Chr.	Peak marker	QTL bordering marker	SMA			IM			CIM		
				LOD	[b]R^2 (%)	Additive effect	LOD	R^2 (%)	Additive effect	[c]LOD	R^2 (%)	Additive effect
qCTS1.1	1	RM490	RM220 - RM10829	-	-	-	-	-	-	6.1*	50.7	-1.15
qCTS3	3	RM218	RM546-RM7	7.3	57.2	-1.8	5.23	45.2	-0.216	4.3*	39.0	-0.54
qCTS11.2	11	RM7283	RM7283-RM3428	-	-	-	3.51	33.3	-1.37	3.6	34.3	-0.84
qCTS11.4	11	RM144	RM254-RM144	4.1	38.1	-0.8	3.42	31.3	1.78	2.9[ns]	27.4	-0.8
qCTS12	12	RM1261	RM27877-RM463	9.6	66.9	2.2	11.41	73.1	2.55	6.5*	52.7	1.58

CTS, cold tolerance at seedling stage. [I]Individual QTL is designed with q indicating QTLs with LOD > 2.5, an abbreviation of the trait name and the chromosome number on the chromosome, suggestive QTLs are indicated in italic. [b]Portion of phenotypic variation explained by the individual QTL. [C]Significant loci after 1000 permutation analysis at $p < 0.05$ with threshold LOD (Logarithm of Odds) of 4.3. *Significant at 5% level of significance. [ns]Null significant showed after significant analysis.

The significant QTLs were located on chromosomes 1, 3 and 12 with marker intervals of RM220 - RM10829, RM546 - RM7 and RM27877 - RM463, respectively. Several previous studies also reported QTLs on chromosomes 1, 3 and 12. Park *et al.* (2013), Liu *et al.* (2013) and Zhi-Juan *et al*. (2010) reported QTLs for seedling stage cold tolerance on chromosome 1 at 2.6 - 4.2 Mb, 0.5 - 4.0 Mb and 0.5 - 4.0 Mb, respectively; similarly Zhi-Juan *et al.* (2010) and Zhang *et al.* (2014) reported QTLs on chromosome 3 at 9.9 -12.4 Mb and 19.8 - 23.1 Mb, respectively; while Han *et al.* (2007), Zhang *et al*. (2013), Andaya and Mackill (2003) and Verma *et al*. (2014) reported QTL (qCTS12) on chromosome 12 at 17.8 - 26.9 Mb, 3.8 - 5.8 Mb, 8.8 - 9.6 Mb and 4.9 - 5.3 Mb, respectively. But the physical positions of QTLs of the present trait were different from those of previously reported QTLs. We mapped *qCTS1.1* at 4.4 Mb - 13.3 Mb on chromosome 1, *qCTS3* at 6.2 Mb – 9.8 Mb on chromosome 3 and *qCTS12* on chromosome 12 at 9.2 Mb - 22.0 Mb. The significant QTLs *i.e. qCTS1.1*, *qCTS3* and *qCTS12* of this study contributed 50.7, 39 and 52.7% to the total phenotypic variation, respectively. QTLs with such higher phenotypic contribution were also reported by many other researchers. For example, *qLT3-1* (35% PV) on chromosome 3 was reported by Fujino *et al.* (2004), similarly *qSCT3-1* (24.5% PV) on chromosome 3 and *qCTS12a* (40.6% PV) on chromosome 12 were reported by Andaya and Mackill (2003). The identified QTLs, *qCTS1.1*, *qCTS3* and *qCTS12* were found tightly linked with RM490, RM218 and RM1261, respectively. The *qCTS1.1* and *qCTS3* showed negative additive effects while *qCTS12* showed positive value, which indicated that the favorable alleles of the former two QTLs for cold tolerance were inherited from donor parent BR18 and that of latter was arisen from susceptible parent BR1.

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