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Short Communication

Identification of QTLs for cold tolerance at seedling stage in rice (*Oryza sativa* L.)

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Abstract

Rice is a cold sensitive plant. During off-season cultivation this crop experiences cold at seedling stage. The present study was based on 122 RILs in F_{13} generation developed by single-seed descent method from a cross between two *indica* cultivars Danteshwari and Dagad deshi. Screening was conducted during December 2012-January 2013 with seven replications at seedling stage. The genotypes exhibited marked variation for cold tolerance, with an average of 3.52 score on 1-9 scale and 0.62 broad sense heritability. Genotypic data of this population was developed using 161 SSR markers. A total of 5 QTLs were identified on chromosome 1, 3, 6, 9 and 12. QTL present on chromosome 9 had major effect with LOD value of 9.53 and located between HvSSR 9-7 to HvSSR 9-19. These QTLs will be helpful to facilitate the development of novel cold tolerant cultivars using molecular breeding.

Key words: Rice (*Oryza sativa* L.), seedling stage, cold tolerance, QTLs

Rice is important crop grown in different sets of conditions. The optimum temperature for seed germination and early seedling growth is from 25 to 35^{o}C. Early seedling stage is important for subsequent growth. In India, particularly eastern India, when rice is grown during *rabi* season, there is a problem of low temperature at seedling stage. Low temperature conditions below 15^{o}C at seedling stage commonly leads to poor germination, seedling stunting, yellowing or withering, reduced tillering and mortality [1-4], which inhibits seedling establishment and eventual yield loss. Developing cold-tolerant genotypes is one of the most effective ways to avoid the low-temperature damage [5]. Lot of variation for cold tolerance in rice has been reported [6, 7]. The inheritance of cold tolerance has been reported to be quantitative in nature [8-10]. This study, therefore, aims to identify QTLs for cold tolerance at seedling stage, which can be subsequently used for MAS based development of cold tolerant genotypes suitable for cultivation in Eastern India.

In present investigation 122 recombinant inbred lines (RILs) developed by single-seed descent method from a cross between two *indica* cultivars Danteshwari and Dagad deshi were evaluated under field condition during December/January 2012-13, in RCBD including Sahabhagi dhan (as susceptible check) in seven replications at Research cum Instructional Farm, IGKV, Raipur (21^{o} 16' N and 81^{o} 36' E at altitude of 289.6 meters above mean sea level). During December-January usually the minimum temperature goes below 15^{o}C and this is the time when farmers also do sowing for up-coming off-season crop. One row of each genotype was sown on raised nursery bed with row length of 75 cm and spacing of 10 cm between rows. A similar screening technique was also used by Hadmani [11]. Lines were scored on 1-9 scale according to SES of rice, IRRI. Observations were recorded when Sahabhagi dhan exhibited the score of 7 or 9, at about 20-25 days old seedlings. Seedlings

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were scored as 1-2 (dark green), 3-4 (light green), 5-6 (yellow), 7-8 (brown) and 9 (dead).

For developing genotypic data based on SSR markers, DNA was extracted from fresh leaf with the help of MiniPrep method [12]. Polymerase chain reaction (PCR) amplification was performed in a total volume of 20 µl and the reaction mixture contained 10 X Assay buffer, 1 mM dNTPs mix, 5 pM forward and reverse primers, 40 ng of template DNA and 1 unit *Taq* polymerase in a Applied Biosystems thermal cycler. After an initial denaturation step of 95°C for 5 min, the amplification was carried out for 34 cycles comprising 1 min each of 94°C, 55°C and 72°C. The final elongation step was extended to 7 min at 72°C followed by 4°C. After the PCR reaction was completed, 5 µl of 6 X loading dye was added to PCR amplicons and 7 µl was resolved on 5% PAGE in a mini-vertical electrophoresis system (CBS scientific, model MGV-202-33). DNA fragments were then stained with ethidium bromide and visualized with a UV transilluminator Bio-rad XLR$^+$ (Fig. 3).

For the purpose of genotyping 830 markers were used, out of which 161 were polymorphic, exhibiting 19.40% polymorphism. Differences among polymorphic markers were observed for allele segregation in RILs. Out of 161 markers, 84 (52.17%) exhibited normal 1:1 segregation at 1% level of significant in χ^2 test, rest which exhibited skewed distribution towards either parents. The marker HvSSR 7-53 produces more female type alleles (80.5%) with 18.7% male. On the other hand RM 277 produces 11.4%, 82.1%. HvSSR 5-51 marker had high A: B ratio (5.5) such skewed distribution for markers has been reported by Cai *et al*. [15]. QTL cartographer 2.5 was used to construct a linkage map with 161 SSR markers covering all 12 rice chromosomes. Position of each marker was assigned based on published literature [13] and website of gramene (http://www.gramene.org). The phenotypic and genotypic data was then analyzed using QTL cartographer 2.5 (composite interval mapping) for identification of QTLs for cold tolerance with a threshold value of 3.0 LOD [14] (Fig. 4).

A perusal of meteorological data during the experimental period (Fig. 1) indicates the exposure of the plant material at low temperature. At this temperature under the field condition, the susceptible check exhibited a sensitivity score of 7 to 9 and the test genotype exhibited differential reactions. Morphological differences such as reduction in plant height, growth, tillering and yellowing of leaf was observed after chilling exposure during two-week growth period. The analysis of variance revealed significant differences in the RILs tested. This trait showed medium heritability (0.62) with high genetic advanced as per cent of mean (56.97), which indicated the presence of additive gene action and selection will be effective. Line numbers 102, 7 and 2 having low cold score, showed tolerance to cold, whereas line numbers 75, 17, 82 and 81 were highly susceptible to cold had 7-9 score. Significantly higher yield of lines 7, 17 and 82 as compare to other RILs, parent and checks (for yield) IR-64/MTU-1010 indicated better agronomical potential of these lines.

The frequency distribution of cold tolerance scores is presented in Fig. 2. The individual RIL lines exhibited a wide range of reaction from 1 to 9 with an average of 3.52. A normal frequency distribution pattern indicated the polygenic inheritance of this trait. Inheritance of cold tolerance has also been earlier reported to be quantitative in nature [8, 10].

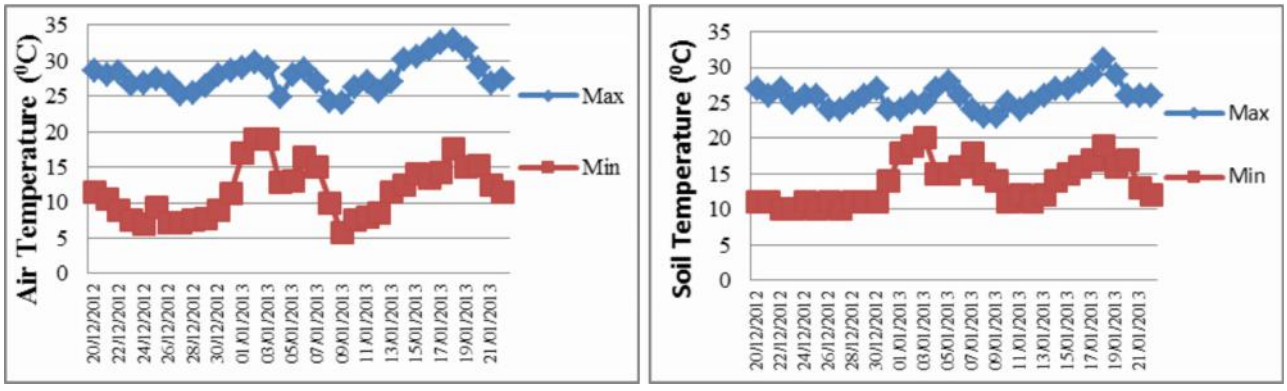

Fig. 1. Minimum and maximum temperature of air and soil during experiment

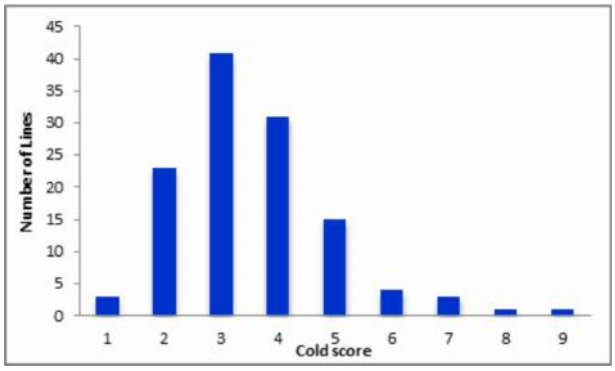

Fig. 2. Frequency distribution of cold tolerance scores based on the overall average

(17.43cM) had LOD score of 4.72. This QTL had negative additive effect, which showed that the allele was transmitted from susceptible parent Danteshwari. Andaya and Mackill [8] also reported QTL on chromosome 6 and 9 for cold tolerance, but the region was different. QTL identified on chromosome 1 between RM 449 and RM 5 had negative additive effect. QTL qCTS-1-b on similar region was also reported by Lou *et al.* [5]. This identified region on chromosome 1 was different than earlier reported QTL by Ranawake *et al.* [16] and qCTB1.1 by Zeng *et al*. [17] on same chromosome. QTL identified on chromosome 3 between HvSSSR 3-35 and HvSSR 3-56 with positive

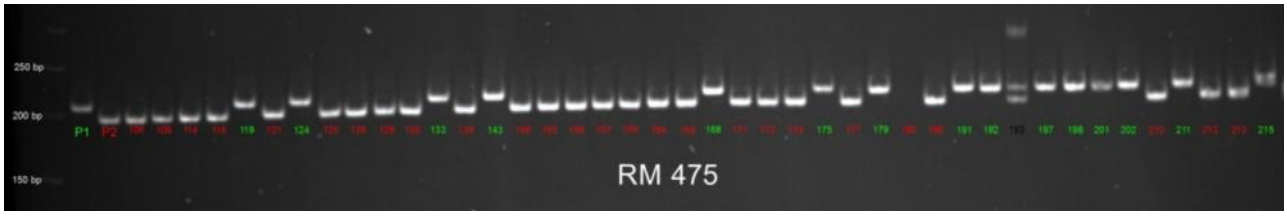

Fig. 3. Banding pattern of Microsatellite primer RM 475

The genotypic data and field based phenotypic data of reaction to cold of RIL population was analyzed using QTL cartographer 2.5. Results from the QTL analysis is presented in Table 1 and Fig. 4. A total of 5 QTLs were identified for cold tolerance on chromosome 1, 3, 6, 9 and 12. A new QTL was identified on chromosome 9 between HvSSR 9-7 (4.35cM) to HvSSR 9-19 (10.50cM) having LOD score of 9.53. This QTLs had positive additive effects (Table 1), indicating that alleles at this loci increase cold tolerance come from tolerant parent Dagad deshi. Another new QTL was identified on chromosome 6 between HvSSR 6-35 (10.82cM) and HvSSR 6-44

Table 1. QTLs for cold tolerance in *rabi* 2012-13 seedling stage

Chr	Interval	LOD	Additive effect
1	RM 449 to RM 5	3.24	–0.28
3	HvSSR 3-35 to HvSSR 3-56	3.00	0.40
6	HvSSR 6-35 to HvSSR 6-44	4.72	–0.54
9	HvSSR 9-7 to HvSSR 9-19	9.53	0.71
12	HvSSR 12-35 to HvSSR 12-40	3.53	–0.35

Chr = chromosome

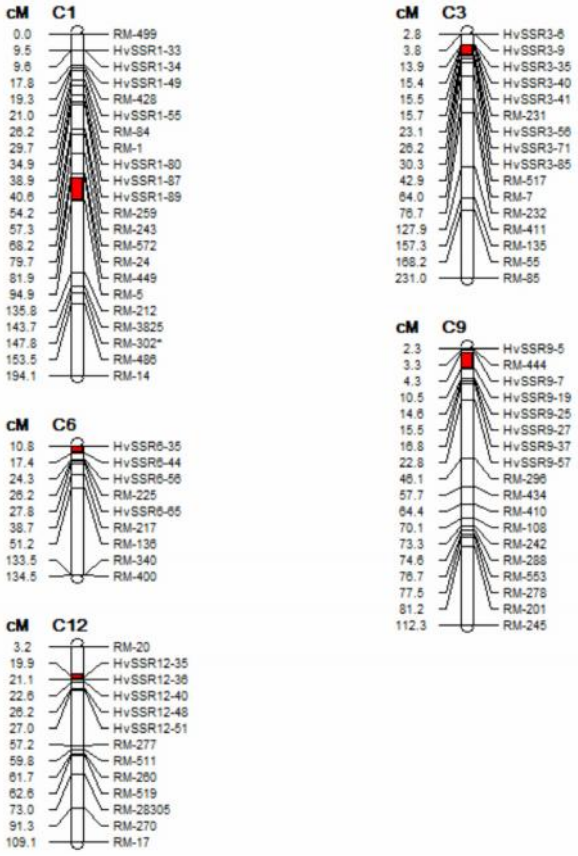

Fig. 4. Genetic map locating all QTLs for cold tolerance

additive effect. Koseki *et al*. [18] and Shirasawa *et al*. [10] also reported QTL on chromosome 3 for cold tolerance. This region of chromosome 3 was different than QTL region identified by Ranawake *et al*. [16] on the same chromosome. QTL identified on chromosome 12 between HvSSR 12-35 and HvSSR 12-40 had negative additive effect. QTL for cold tolerance has been reported on chromosome 12 by others [8, 16, 19]. All these QTLs, together explained 62 per cent variability. This information could facilitate the development of novel cold tolerant cultivars using molecular breeding such as MAS.

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