Rice Science, 2006, 13(2): 93-98
http://www.ricesci.cn; www.ricescience.org

QTL Mapping of Low Temperature on Germination rate of Rice

CHEN Liang[1], LOU Qiao-jun[1, 2], SUN Zong-xiu[3], XING Yong-zhong[2], YU Xin-qiao[1], LUO Li-jun[1, 2]
([1]*Shanghai Agrobiological Gene Center, Shanghai 201106, China*; [2]*College of Plant Science and Technology, Huazhong Agricultural University, Wuhan 430070, China*; [3]*China National Rice Research Institute, Hangzhou 310006, China*)

Abstract: To investigate the low temperature on germination capacity (LTG) a double haploid rice (DH) population with 198 lines derived from anther culture of F_1 hybrid with indica line Zhenshan 97B and a perennial japonica line AAV002863 was used to construct a linkage map with 140 SSR markers. The germination rate in Zhenshan 97B and AAV002863 was 79.7% and 30.1%, while in DH population it ranged from 0 to 100% at 15℃ after 6 days. Quantitative trait loci (QTLs) controlling low temperature germinability were identified on chromosomes 3 and 10. The percentage of observed phenotypic variance attributed to *qLTG-3* and *qLTG-10* was 12.6% and 12.9%, respectively. Allele from Zhenshan 97B increased the LTG at *qLTG-3* region, while allele from AAV002863 increased the LTG at *qLTG-10* region. One pair of epistatic interaction was detected between loci on chromosomes 3 and 10. The main-effect of QTL on chromosome 10 was also involved in epistatic interaction.
Key words: rice; low temperature germinability; quantitative trait locus

Rice is a low temperature-sensitive crop especially during seedling, tillering, panicle development and flowering stages, which can easily be injured by cold treatment. Most of the high-yielding and high quality varieties can not be used in direct sowing because of low germination rate at low temperature (LTG). The reduction in seedling growth of rice due to low temperature is one of the major problems in tropical and subtropical areas at high altitude as well as in areas where cold mountain water is used for irrigation. In such areas, water temperature during sowing is below 15℃, whereas the optimum range for germination and early seedling growth of rice is 25 to 35℃. The delay in seedling emergence due to cold water greatly increases seedling mortality and causes serious decreases in yield and increases competition with weed. In many Asian countries, the direct seedling culture has become increasingly important in rice growing areas. Therefore, vigorous germination at low temperature is an important character for establishment of stable seedlings in direct seedling culture, where rice is sown directly into flooded fields.

A wide range of variation for LTG has been observed in rice varieties from Japan, Europe, China, Russia and other regions [1]. However, despite the substantial amount of genetic variation for LTG in rice, it has not been an easy task to improve the level of LTG in rice breeding programs due to lack of clarification of genetic basis of LTG and quantitative inheritance. The phenotypic correlation between LTG and several morphological marker genes, d_2, *wx*, d_6 and *I-Bf*, located on chromosomes 1, 6, 7 and 9, respectively, were previously reported [2]. Based on the development of molecular biology, several studies on QTL mapping of LTG have been reported [3-8]. Using 81 recombinant inbred lines derived from a cross between Kinmaze (japonica variety) and DV85 (indica variety), five QTLs for LTG have been identified [3]. Three QTLs controlling LTG on chromosomes 3 and 4 were identified using 122 backcross-inbred lines derived from a cross between temperate japonica varieties, Italica Livorno and Hayamasari [4]. Five QTLs for LTG located on chromosomes 2, 4, 5 and 11 were detected by crossing between japonica Nipponbare and indica Kasalath [5]. In addition, Teng et al [6] detected two QTLs for LTG on chromosomes 4 and 9 with the DH population derived from ZYQ8/JX17, while Wan et al [7] detected putative QTLs for LTG on chromosomes 3, 6 and 11 using BC_1F_1 lines derived from USSR5/Guangjie 9.

Received: 21 December 2005; **Accepted:** 25 March 2006
Corresponding author: LUO Li-jun (lijun@sagc.org.cn)

Suh et al [8] found a primary mapping of the LTG QTLs on chromosomes 5, 6 and 11 using BC_1F_1 lines derived from Milyang 23 // Milyang 23 / Hapcheonaengmi 3. Moreover, studies on QTL mapping of rice tolerance against low temperature at the early seedling and booting stages have been reported [9-14].

The identification of the number and magnitude of gene effects may contribute to a better understanding of the genetic control of LTG and to facilitate the rapid development of rice varieties with vigorous LTG. The objective of the present research is to identify QTLs for LTG using a DH population derived from an indica variety Zhenshan 97B and a perennial japonica variety AAV002863 that can overwinter in southern China under natural field conditions. LTG of the DH population was evaluated with germination percentage of each DH line at 15℃ for 4 days.

MATERIALS AND METHODS

Plant materials

A DH population with 198 lines derived from F_1 anther culture of Zhenshan 97B / AAV002863 was used for evaluating LTG in rice.

Evaluation of LTG

To break up the dormancy, the seeds of the DH lines and parents were incubated at 50℃ for 3 d. One hundred seeds of each line and the parents were placed on filter paper moistened with distilled water in a plastic germination box with the dimension of 12.5 cm × 12.5 cm × 2.0 cm. The data for LTG was recorded at 15℃ after 6 d of treatment. After 10 d, the seeds were moved into an incubator and the germination rate was again calculated after 4 d of incubation at 30℃. It could be deduced that the DH lines were free from secondary dormancy if more than 80% of the seeds germinated.

SSR analysis

Total DNAs of the DH lines and the two parents were extracted using CTAB method [15]. SSR markers were synthesized according to the literatures [16-19]. PCR was performed in a 20 μL volume containing 7 μL DNA template with 25 ng genomic DNA, 2 μL 10 × buffer, 1 μL forward primer and 1 μL reverse primer at a concentration of 5 μmol/L, 1 μL 2 mmol/L dNTPs, 0.2 μL (5 U/μL) *Taq* polymerase and 7.2 μL ddH_2O. The PCR was programmed as follows: 94℃ for 5 min, followed by 35 cycles of 94℃ for 1 min, 55℃ for 1 min, 72℃ for 2 min, and a final extension at 72℃ for 7 min. PCR products were analyzed in 3% agarose gel or 5% polyacrylamide gel electrophoresis using a simple silver staining method [20].

Linkage mapping and QTL analysis

Linkage map was constructed by the use of Mapmaker/EXP3.0 with the SSR genotypes of the DH population. QTL was identified by the use of QTLMapper 1.0 based on a mixed model approach [21]. A threshold of $P \leqslant 0.005$ was used to select QTL with significant effect. For digenic epistatic QTL detection, the threshold was $P \leqslant 0.001$. Contribution rate (R^2) was estimated as the percentage of variance explained by each locus or epistatic pair in relation to total phenotypic variance, while the main effect QTL was named following the popular nomenclature [22].

RESULTS

Linkage map construction

From 533 SSR markers, 232 markers displayed polymorphism between Zhenshan 97B and AAV002863, 140 polymorphic markers evenly distributed on 12 chromosomes were selected for genotypic and construction of genetic map with an average distance of 9.7 cM between markers. The polymorphic distribution of markers on each chromosome is given in Table 1.

Marker segregation analysis

All the 140 microsatellite loci involved in construction of the genetic map were tested for segregation distortion. Distortion was detected using the χ^2-test for goodness of fit to expected allelic frequency of 1∶1 (indica allele∶ japonica allele) (Table 2 and Fig. 1). The actual allele frequency of the DH population was calculated at 0.52 and 0.48 for the Zhenshan 97B and AAV002863, respectively.

Table 1. Number of microsatellite markers surveyed and used for map construction per chromosome, the degree of polymorphism and chromosome length in cM for the rice linkage map.

Chromosome	No. of detected markers	No. of polymorphic markers	Percentage of polymorphism (%)	No. of markers for map construction (%)	Chromosome length (cM)
1	82	29	35.4	18	203.1
2	65	33	50.8	16	134.6
3	58	24	41.4	15	150.9
4	33	19	57.6	11	137.9
5	45	24	53.3	8	73.0
6	56	31	55.4	12	89.6
7	41	20	48.8	12	71.8
8	47	16	34.0	14	155.6
9	31	7	22.6	7	67.0
10	23	11	47.8	10	73.1
11	28	10	35.7	9	80.5
12	24	8	33.3	8	119.9
Total	533	232	43.5	140	1355.9

Table 2. Number and ratio of distorted markers on individual chromosome.

Chromosome	No. of surveyed SSR markers	No. of markers for distorted segregation	No. of markers skewed towards the female parent	No. of markers skewed towards the male parent
1	18	7	2	5
2	16	12	11	1
3	15	8	3	5
4	11	1	1	0
5	8	5	0	5
6	12	11	11	0
7	14	6	0	6
8	14	6	5	1
9	7	5	5	0
10	10	7	1	6
11	9	4	0	4
12	8	1	1	0
Total	140	73	40	33

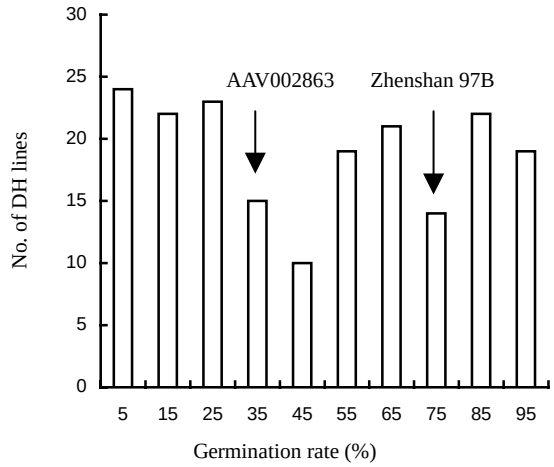

Fig. 1. Frequency distribution of germination rate at 15℃ for 6 days in the DH population.

Of the 140 mapped loci, 29.3% were skewed in favor of the Zhenshan 97B allele while 23.6% were skewed towards the AAV002863 allele. The allelic frequency for Zhenshan 97B ranged from 0.228 to 0.826, while each chromosome has distorted markers.

Phenotypic variation and QTL detection

LTG of the DH population was evaluated at 15℃ for 6 d. The germination rate of Zhenshan 97B and AAV002863 was 79.7% and 30.1%, respectively, while the germination rate of the DH population ranged from 0% to 100% with a mean of 48.3%, showing a continuous distribution and transgressive segregation (Fig. 1). The average germination rate of the DH population increased to 94.0% after the seeds were shifted into an incubator at 30℃ for 4 d.

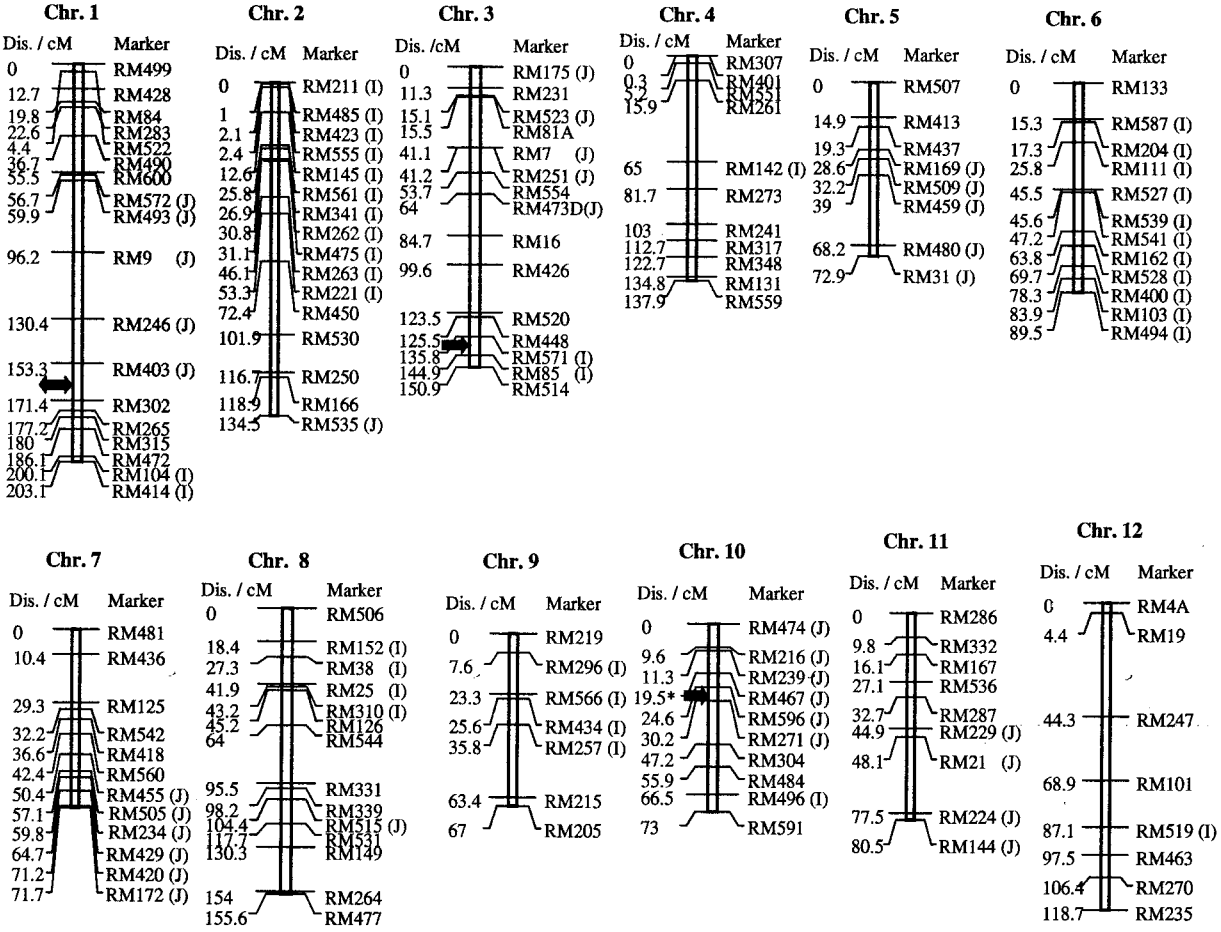

Fig. 2. Microsatellite linkage map developed using 198 DH lines derived from an indica × japonica cross and the chromosomal regions showing markers with distorted segregation, and chromosomal location of putative QTLs detected for low temperature germinability.

(I) indica allele from Zhenshan 97B; (J) japonica allele from AAV002863. ➡ Main-effect QTLs; ⬌ Epistatic loci; * indicates that this main-effect QTL also interacts.

Table 3. Putative QTLs and their additive effects for LTG in the rice DH population.

Locus	Chromosome	Marker interval	LOD value	Additive	R^2/%
qLTG-3	3	RM85–RM514	5.94	-11.86	12.60
qLTG-10	10	RM596–RM271	4.20	11.99	12.88

It indicated that the LTG of the DH population was not affected by the secondary dormancy.

Two QTLs for LTG were detected on chromosomes 3 and 10 (Table 3, Fig. 2). *qLTG-3* located on chromosome 3 between markers of RM85 and RM514 explained 12.60% of total phenotypic variance with a LOD value of 5.94. The allele from Zhenshan 97B increased the germination rate. The *qLTG-10* located between RM596 and RM271 on chromosome 10, accounting for 12.88% of phenotypic variation. The allele from AAV002863 increased germination rate.

Only one pair of epistatic QTL was detected on chromosomes 1 and 10, accounting for 5.96% of phenotypic variance (Table 4). The positive interaction between these two loci indicated that the parental genotype could increase the germination rate at low temperature. The main-effect QTL, *qLTG-10* was also involved in epistatic interaction.

Table 4. Epistatic loci for low temperature germinability in the rice DH population.

Chromosome	Marker interval	Chromosome	Marker interval	LOD Value	AAE [a]	R^2/%[b]
1	RM403–RM302	10	*RM596–RM271	6.08	8.26	5.96

[a] Additive × Additive effect (AAE): positive values indicate that the two-locus genotypes being the same as those in parent take the positive effects, while the two-locus recombinants take the negative effects. The case of negative value is just the opposite. [b] Variance explained by each pair of epistatic loci. * The locus located within the interval simultaneously has its additive main-effect.

DISCUSSION

The proper temperature tested to investigate cold tolerance during rice germination is a prerequisite to correctly appraise the LTG. Sasaki et al [2] found that the result of the LTG under 15℃ for 10 d were the same as that under 13℃ and was highly correlated with that under 10℃ and suggested 15℃ as a test temperature for investigation of cold tolerance during germination period. Although in most of the studies for LTG the recommended test temperature is 15℃, but the days for low temperature treatment varied in different studies [3-5]. In present work, 15℃/6 d was used to evaluate the LTG of the DH population as the germination rate between the two parents and among the DH population showed the greatest difference during that period. To apply QTL mapping results in breeding program, the test days for low temperature germination should be shorter. In order to eliminate the effect of seed maturity, seed storage and seed dormancy, the seed germination rate under normal temperature must be tested before the LTG test. Moreover, seed germination test under normal temperature is also required after LTG test to avoid secondary dormancy during germination.

High germination rate at relatively low temperature is an essential characteristic for rice direct sowing culture in many countries such as, northern China, northern Japan and Korea. Several researchers [3-8] had found some QTLs controlling LTG. Four reports [3, 5, 7-8] detected QTL for LTG on chromosome 11, *qLTG-11* was identified at the same region by Hou et al [3] and Miura et al [5], and was also similar to the locus of *qLTG-11* detected by Wan et al [7]. Moreover, *qLTG-6* detected by Hou et al [3], Wan et al [7], Suh et al [8] was located at the same region. In this study, two QTLs underlying LTG were identified on chromosomes 3 and 10. The QTLs detected on *qLTG-3* during our study is similar to the result reported by Fujino et al [4] but different to the findings of Wan et al [7]. Previously, no QTL was detected on chromosome 10, whereas the identification of *qLTG-10* in this study showed a new locus for LTG. In conclusion, it is very practical to develop rice varieties with high germination rate at low temperature by pyramiding alleles that could increase the LTG from different donors through marker-assisted selection in rice breeding program.

ACKNOWLEDGEMENTS

This research was supported by the Youth Fund of the QIMINGXING Project of the Shanghai Committee of Science and Technology (No. 04qmx1458) and Key National Basic Research Developments Program of the Ministry of Science and Technology, P. R. China (No. 2004CB117204).

REFERENCES

1 Kotaka S, Abe N. The varietal difference of germinability at low-temperature in rice varieties and the testing method for the percentage establishment of seedlings. *J Agric Sci Tokyo*, 1988, **43**: 165-168.

2 Sasaki T, Kinoshita T, Takahashi M. Estimation of the number of genes in the germination ability at low temperature in rice. *J Fac Agric Hokkaido Univ*, 1973, **197**(357): 301-312.

3 Hou M Y, Wang C M, Jiang L, Wan J M, Hidshi Y, Atsushi Y. Inheritance and QTL mapping of low temperature germinability in rice (*Oryza sativa* L.). *Acta Genet Sin*, 2004, **31**(7): 701-706.

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

5 Miura K, Lin S Y, Yano M, Nagamine T. Mapping quantitative trait loci controlling low-temperature

germinability in rice (*Oryza sativa* L.). *Breeding Sci*, 2001, **51**: 293-299.

6 Teng S, Zeng D L, Qian Q, Ysaufumi K, Huang D N, Zhu L H. QTL analysis of rice low temperature germinability. *Chinese Sci Bull*, 2001, **46**(21): 1800-1803.

7 Wan J, Tamura K, Sakai M, Imbe T. Linkage analysis of low temperature germinability in rice (*Oryza sativa* L.). *Breeding Sci*, 1999 (Suppl 2): 122. (in Japanese)

8 Suh J P, Ahn S N, Moon H P, Suh H S. QTL analysis of low temperature germinability in a Korean weedy rice (*Oryza sativa* L.). *Rice Genet Newsl*, 1999, **16**: 53-55.

9 Liu F X, Sun C Q, Tan L B, Fu Y C, Li D J, Wang X K. Identification and mapping of quantitative trait loci controlling cold-tolerance of Chinese common wild rice (*O. rufipogon* Griff.) at booting to flowering stages. *Chinese Sci Bull*, 2003, **48**(19): 2068-2073.

10 Qian Q, Zeng D L, He P, Zheng X W, Chen Y, Zhu L H. The QTL analysis of seedling cold tolerance in a double haploid population derived from anther culture of hybrid of indica/japonica. *Chinese Sci Bull*, 2000, **45**(5): 448-453.

11 Yan C J, Li X, Cheng Z K, Yu H X, Gu M H, Zhu L H. Identification of QTL for cold tolerance at early seedling stage in rice (*Oryza sativa* L.) via RFLP marker. *Chinese J Rice Sci*, 1999, **13**(3): 134-138. (in Chinese with English abstract)

12 Andaya V C, Mackill D J. Mapping of QTLs associated with cold tolerance during the vegetative stage in rice. *J Exp Bot*, 2003, **54**(392): 2579-2585.

13 Andaya V C, Mackill D J. QTLs conferring cold tolerance at the booting stage of rice using recombinant inbred lines from a japonica × indica cross. *Theor Appl Genet*, 2003, **106**(6): 1084-1090.

14 Saito K, Hayano-Saito Y, Maruyama-Funatsuki W, Sato Y, Kato A. Physical mapping and putative candidate gene identification of a quantitative trait locus *Ctb1* for cold tolerance at the booting stage of rice. *Theor Appl Genet*, 2004, **109**(3): 515-522.

15 Murray M G, Thompson W F. Rapid isolation of high molecular weight plant DNA. *Nucl Acids Res*, 1980, **8**: 4321-4325.

16 Temnykh S, Park W D, Ayres N, Cartinhour S, Hauck N, Lipovich L, Cho Y G, Ishii T, McCouch S R. Mapping and genome organization of microsatellite sequences in rice (*Oryza sativa* L.). *Theor Appl Genet*, 2000, **100**: 697-712.

17 Akagi H, Yokozeki Y, Inagaki A, Fujimura T. Microsatellite DNA markers for rice chromosomes. *Theor Appl Genet*, 1996, **93**: 1071-1077.

18 Chen X, Temnykh S, Xu Y, Cho Y G, McCouch S R. Development of a microsatellite framework map providing genome-wide coverage in rice (*Oryza sativa* L.). *Theor Appl Genet*, 1997, **95**: 553-567.

19 Temnykh S, DeClerk G, Lukashova A, Lipovich L, Cartinhour S, McCouch S R. Computational and experimental analysis of microsatellites in rice (*Oryza sativa* L.): Frequency, length variation, transposon associations, and genetic marker potential. *Genome Res*, 2001, **11**: 1441-1452.

20 Xu S B, Tao Y F, Yang Z Q, Chu J Y. A simple and rapid methods used for silver staining and gel preservation. *Heriditas*, 2002, **24** (3): 335-336. (in Chinese with English abstract)

21 Wang D L, Zhu J, Li Z K. Mapping QTLs with epistatic effects and QTL × environment interactions by mixed linear model approaches. *Theor Appl Genet*, 1999, **99**: 1255-1264.

22 McCouch, S R, Cho, Y G, Yano M. Report on QTL nomenclature. *Rice Genet Newsl*, 1997, **14**: 11.

23 Jin R Z. Genetics and evaluation of cold tolerance of rice in Japan. *Rice Rev & Abstr*, 1990, **9**(3): 1-5. (in Chinese)