NOTES

QTL analysis of rice low temperature germinability

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Abstract **A double haploid population, derived from anther culture of F_1 hybrid between a typical *indica* and a *japonica* (ZYQ8/JX17), has been used to investigate the low temperature germinability (LTG) at 15℃. The low temperature germinability of two parents was significantly different. In 6—11 d, the germination percentage of ZYQ8 was higher than that of JX17. In 12—16 d, the germination percentage of JX17 was higher than that of ZYQ8. The quantitative trait loci (QTLs) of every day for low temperature germinability have been mapped based on a molecular linkage map constructed from this population. In 8—11 d, qLTG-9 was identified in C397B-RZ617B on chromosome 9, the additive effect was positive, showing that the allele from JX17 could increase low temperature germinability. In 12—16 d, qLTG-4 was mapped between RG908 and CT563 on chromosome 4, the additive effect was negative, showing that the allele from ZYQ8 could increase low temperature germinability. These two QTLs were detected at different stages, showing the complexity of the mechanism of low temperature germinability.**

Keywords: Indica and japonica subspecies, DH population, low temperature germinability, QTL detection, dynamical analysis.

With the development of economy, the planting area of direct sowing rice is getting larger and larger because it saves time, labor and water. Low temperature germinability is very important for the direct sowing rice. However, rice is a temperature-sensitive crop, which can easily be hurt by cold in the periods of shoot, seedling, panicle development and flowering. Many high-yield and good-quality varieties cannot be used as direct sowing rice because of low germinability in low temperature. This limits the development of direct sowing rice. Genetics and breeding study of low temperature germinability has been a new hot point[1].

Systemic study on the genetic basis of rice cold tolerance had been undertaken. Yan et al.[2] detected one QTL for cold tolerance by using a double haploid (DH) population from anther culture of F_1 hybrid between an *indica* rice Nanjing 11 and a *japonica* rice Ballia. Qian et al.[3] identified four QTLs for seedling cold tolerance by using a double haploid (DH) population from an *indica/japonica* cross (ZYQ8/JX17). Zeng et al.[4] found several QTLs for cold tolerance in panicle development period. However, the genetic basis of low temperature germinability of rice was still unclear. It was reported that the heritability of cold tolerance in germination period of rice was high, so it could be selected in the low generation, and the cold tolerance in germination period is a quantitative trait controlled by genes with additive effect and dominant of low degree[5]. It was reported that cold tolerance in germination period was a dominant trait, and controlled by about 5 additive genes[5].

Recently, molecular markers, such as RFLP, AFLP and SSR have been developed. A number of rice molecular linkage maps have been constructed. Accurate analysis of the number of quantitative trait loci (QTLs), the position of these loci on chromosome and their genetic effects have been realized after using the permanent population, such as double haploid (DH), and recombinant inbred (RI) population, and their molecular linkage map. In fact, molecular markers have been widely used in identification of QTLs for many important agronomic traits[3,6—8]. In this study, DH population derived from anther culture of ZYQ8/JX17, a typical indica/japonica hybrid was used. The germination percentage of every DH line at 15℃ was investigated every day, and QTLs for low temperature germinability in a dynamic mode were analyzed.

1 Materials and methods

(ⅰ) Materials. A DH population derived from anther culture of F_1 hybrids between ZYQ8, a typical *indica* variety, and JX17, a typical *japonica* was used. There were 127 DH lines in this population[3].

(ⅱ) Low temperature germinability evaluation. The same maturity seeds of DH lines and the parents, which were stored 3 months in open conditions under room temperature and with the dormancy broken at 50℃, were first sterilized by dipping in 20% hypochlorite sodium for 0.5 h, then washed three times with distilled water. Two lays of filter paper were spread in a culture dish. The seeds were put upon the filter paper. 5 mL of distilled water per culture dish was added. The culture dishes were incubated in growth cabinet, in which the temperature was maintained at 15℃. Three duplications with 50 seeds per duplication were set. From the 2nd day to the 16th day, the germination percentage was investigated every day. Means of the replications were used in data analysis.

(ⅲ) QTL analysis. Based on the constructed linkage map of this DH population, interval QTL mapping was conducted to analyze the QTLs for every day germination percentage by using the software of Mapmaker/QTL ver 1.1. The presence of a QTL was determined with a LOD threshold of 2.0. LOD>2.0 indicated that the highest LOD score position in the interval was a

QTL for the trait. The variation expression and additive effect of each QTL were also calculated. QTL nomenclature followed that of McCouch et al.[9].

2 Results

(ⅰ) Performance of low temperature germinability in the parents. The germination process at 15℃ of the parents is shown in fig 1. It was found that ZYQ8 began germinating at the 6th day, and JX17 began germinating at the 7th day. The germination percentage of ZYQ8 increased slowly, while the dynamic germination curve of JX17 was like "S". The germination percentage increased quickly in 9—12 d. The germination percentage of ZYQ8 was higher than that of JX17 from 6—11 d, while from 12—16 d, JX17 was higher than ZYQ8.

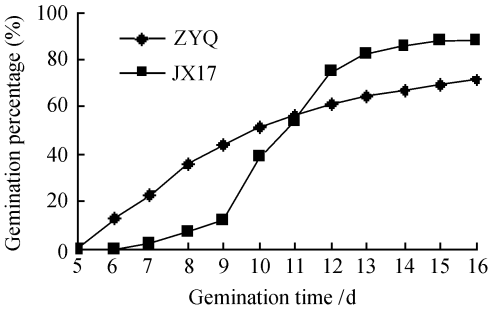

Fig. 1. The dynamical processes of germinations of JX17 and ZYQ8.

(ⅱ) Dynamic variation of low temperature germinability of DH population. Fig. 2 shows dynamical variation of everyday germination percentages of DH lines. For the whole DH population, the longer the germination time was, the higher the germination percentage would be. The distributions of germination percentage of every day were all continuos, and there were some transgressive types. So, it was suitable for QTL mapping.

(ⅲ) QTL analysis. A molecular linkage map had been constructed by using this population. This map contains 234 markers evenly distributed over all 12 rice chromosomes and suitable for QTL analysis[3, 10].

Results of dynamical QTL analysis for low temperature germinability are listed in table 1. A total of 2 QTLs for low temperature germinability, qLTG-4 and qLTG-9, were identified on chromosomes 4 and 9 respectively (fig. 3). By dynamic analysis, qLTG-9, located in C397B-RZ617B on chromosome 9, was detected in 8—11 d with the maximum LOD score on the 9th day. Their additive effects were all positive, indicating that *japonica* allele from JX17 increased germinability. qLTG-4 located in RG908-CT563 on chormosome 4 was detected in 12—

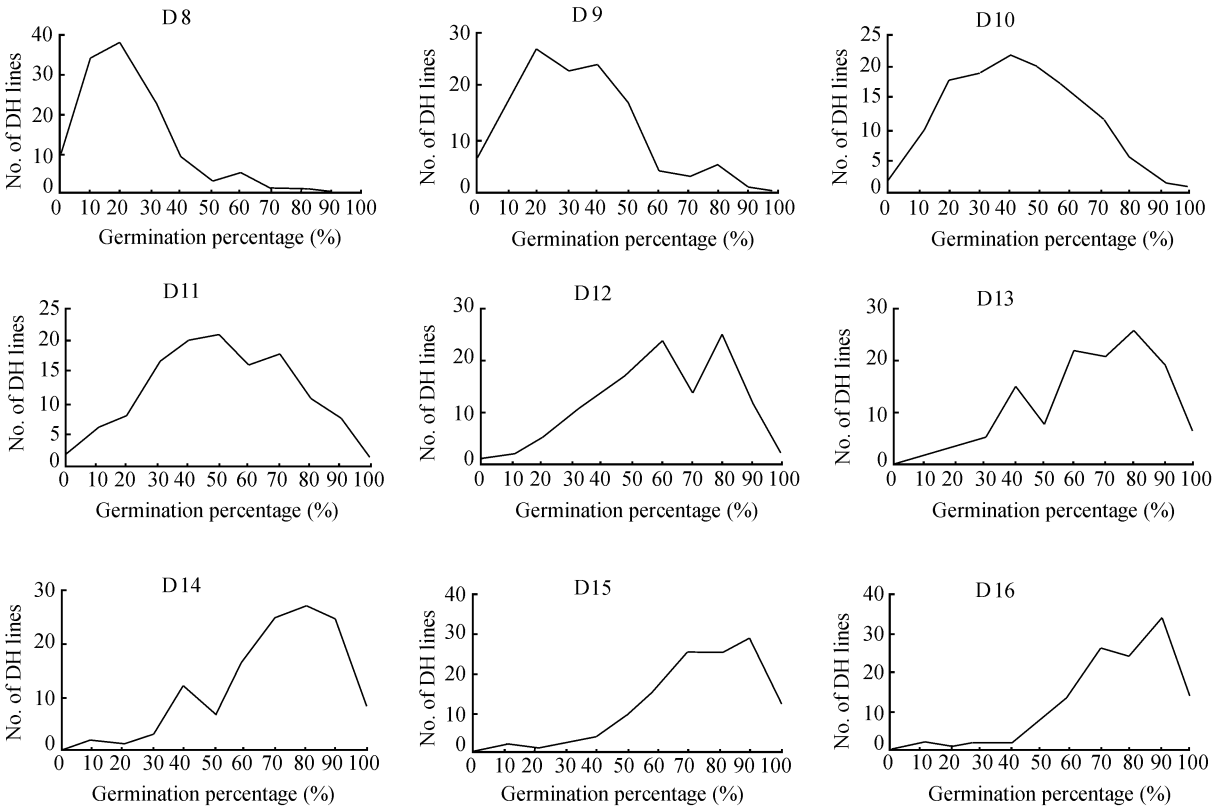

Fig. 2. The dynamic variation of the distribution of germination percentage in low temperature of DH population.

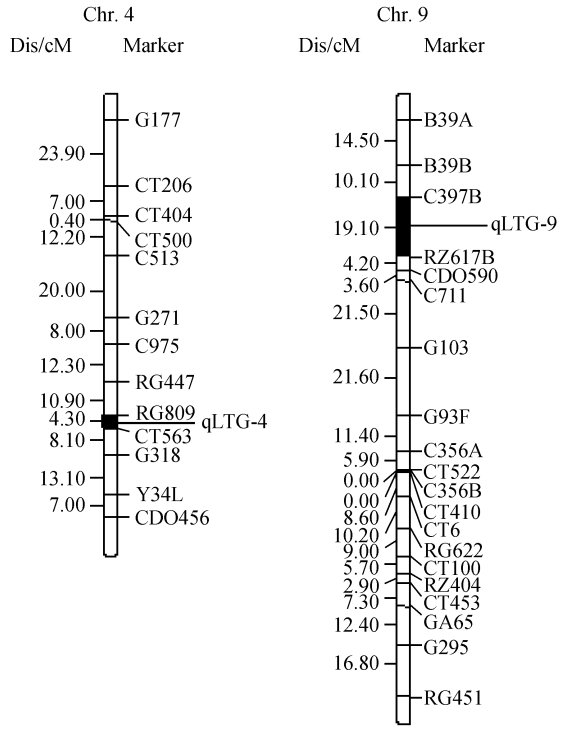

Fig. 3. QTLs for low temperature germinability in the ZYQ8/JX17 DH population.

16 d with the maximum LOD score on the 13th day. Their additive effects were all negative, indicating that indica allele from ZYQ8 increased germinability (table 2).

Table 1 QTLs of maximum LOD score for low temperature germinability[a)]

Locus	Chromosome	Marker interval	LOD score	Variation (%)	Additive effect
qLTG-4	4	RG908-CT563	2.85	11.1	15.2
qLTG-9	9	C397B-RZ617B	2.93	12.6	–13.1

a) QTLs nomenclature follows that of McCouch et al.[9]

Table 2 QTLs for low temperature germinability by dynamical analysis[a)]

	D8	D9	D10	D11	D12	D13	D14	D15	D16
qLTG-4	–	–	–	–	+*	+	+	+	+
qLTG-9	+	+*	+	+	–	–	–	–	–

a) +, QTL observed; –, no QTL observed; * maximum LOD score.

(iv) Genotype analysis of the rice DH lines showing transgressive segregation in low temperature germinability. 7 transgressive segregated DH lines were selected for analyzing their low temperature germinability and genotypes (see table 3). The germinability of lines 060, 067, DH27 and 014 was higher than the high germinability parents in all time (in 8—11 d, ZYQ8 was high germinability parent while JX17 was low germinability parent; in 12—16 d, just the contrary). Genotype analysis showed that these 4 DH lines all had two alleles of high germinability. The germinability of lines 063, 066 and 012 was lower than the low germinability parent in all time. Genotype analysis showed that these 3 DH lines had no high germinability alleles. The wide segregation of the cold tolerance in the DH population was the results of gene recombination. High germinability lines would be obtained if high germinability alleles could be pyramided. This provides a rich source for rice low temperature germinability breeding.

3 Discussion

The germinability of rice seeds is not only affected by the environment conditions such as temperature, but also affected by the maturity, the dormancy, and the storage character of the seeds. The dormancy and the storage character of seeds of the parents used in the present study are significantly different. So, the hereditary variation of low temperature germinability includes the hereditary variation of cold tolerance in germination period and the hereditary variations not related to cold tolerance but affecting the germinability, of the maturity, the dormancy and the storage character of the seeds. To eliminate the hereditary variations of these factors, the dormancy broken seeds with the same maturity and short storage time were used in this study. The results of this study reflected the hereditary variation of cold tolerance in germination period on the whole.

In this study, two QTLs for low temperature germinability were detected on chromosomes 4 and 9, respectively. Previously, Qian Qian et al.[3] detected 4 QTLs for seedling cold tolerance by using the same DH population. They were on chromosomes 1, 2, 3, and 4, respectively. The distance between the QTL on chromosome 4 and qLTG-4 was about 30 cM. Yan et al.[2] detected 1 QTL for early seedling cold tolerance, which was on chromosome 7. These results showed that genes for low temperature germinability and cold tolerance at early seedling or seedling stage were different.

The proper test temperatures for investigation of cold tolerance in germination period of rice were different in different reports, as reviewed by Jin[5]. The range was from 5℃ to 14℃, and mostly 10℃. Matsuda, Nakamura and Nagamatsu et al. proposed to use 15℃ as the test temperature. Lee found that the difference of germinability between varieties was most significant under 10℃. Sasaki found that the results of 15℃ after 10 d were the same as that of 13℃, and highly correlated with the results of 10℃. He proposed to use 15℃ as test temperature for investigation of cold tolerance in germination period[5]. In this study, 15℃ was used as the test temperature. In the previons studies F_2-F_3 populations were used to study the genetic basis of germinability under low temperature[5]. In this study, a DH population, which is stable in traits and completely identical in genetic back ground within each DH line, was used. This made the results more accurate and credible.

Table 3 Differences in low temperature germinability of 7 DH lines

Lines	Germination percentage (%)								
	D8	D9	D10	D11	D12	D13	D14	D15	D16
JX17	7.3	12.2	39.0	53.7	75.6	82.9	85.4	87.8	87.8
ZYQ8	35.9	43.6	51.3	56.4	61.5	64.1	66.7	69.2	71.8
063	0.0	0.0	2.4	2.4	2.4	2.4	2.4	2.4	4.8
066	0.0	0.0	0.0	0.0	0.0	4.3	4.3	6.5	8.7
012	5.0	10.0	15.0	15.0	20.0	25.0	25.0	25.0	25.0
060	31.9	51.1	72.3	80.9	89.4	89.4	89.4	89.4	93.6
067	53.1	69.4	77.6	83.7	85.7	89.8	89.8	91.8	93.9
DH27	59.5	75.7	81.1	81.1	83.8	91.9	91.9	91.9	94.6
014	59.1	72.7	79.5	86.4	93.2	95.5	95.5	95.5	95.5

Several indexes for rice germinability under low temperature have been proposed[5]. Nakamura, Nagamatsu, Kasanar used the germination percentage or average germinating day before germination test terminated. Harashima used the days for 25%, 50%, and 75% germination percentage to show the germinating rate. Gassnar preferred to use germination coefficient. Sasaki found that germination coefficient of 10 d under 15℃ was the best. However, these tests only used one day's germination percentage, average germination day or germination coefficient. Their results were obtained from static analysis. In fact, germination under low temperature was a dynamic proceed, so all genes for this dynamic process should be analyzed. Dynamic QTL analysis was undertaken in this study in order to get more accurate results.

By dynamic analysis, two QTLs controlling the low temperature germinability were detected at different time. qLTG-9 mainly affected the low temperature germinability in 7—11 d, and allele from JX17 increased the germinability. In 12—16 d, qLTG-4 mainly affected the low temperature germinability, and allele from ZYQ8 increased the germinability. The 11th d was the boundary of germination ratio of the parents. The germinability of ZYQ8 was higher than that of JX17 from 6 to 11 d, while from 12 to 16 d, JX17 was higher than ZYQ8. The results of dynamic QTL analysis of DH population accorded with the appearance of the parents, indicating that different genes affected low temperature germinability in different time. Some transgressive lines with low temperature germinability were found in this DH population. By analyzing their genotypes of QTLs for low temperature germinability, we found the lines pyramiding two alleles of high germinability, their germinability were higher than the high germinability parent in all time; wherease the germinability of the lines that had no high germinability alleles was lower than the low germinability parent in all time. This indicated that QTL for low temperature germinability detected in this study could be used in molecular breeding of low temperature germinability of rice. Varieties with high germinability in low temperature could be obtained after pyramiding and recombination of high germinability alleles though indica-japonica crossing. This will help to solve the problem of low temperature germination of direct sowing rice.

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