Detection of QTL for Cold Tolerance at Bud Bursting Stage Using Chromosome Segment Substitution Lines in Rice (Oryza sativa)

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Abstract: The cold tolerance at the bud bursting stage (CTB) was evaluated at 5°C by using a set of 95 chromosome segment substitution lines (CSSLs) derived from an indica rice 9311 and a japonica rice Nipponbare with a genetic background of 9311. The result showed that six CSSLs had slightly stronger effect on CTB than 9311. Total four quantitative trait loci (QTLs) for CTB were preliminary mapped on chromosomes 5 and 7 by substitution mapping. qCTB-5-1, qCTB-5-2 and qCTB-5-3 were mapped in the region of RM267–RM1237, RM2422–RM6054 and RM3321–RM1054, which were 21.3 cM, 27.4 cM and 12.7 cM in genetic distance on rice chromosome 5, respectively. qCTB-7 was mapped in the region of RM11–RM2752, which was 6.8 cM in genetic distance on rice chromosome 7.

Key words: chromosome segment substitution lines; cold tolerance; bud bursting stage; substitution mapping; rice

Rice (Oryza sativa L.) is a warm-season crop in tropics and subtropics, and it is sensitive to low temperature. Therefore, the yield loss caused by low temperature has become a world-wide problem in many rice growing regions of high latitude and altitude. Cold injury may happen in all developmental stages in rice growth including the sprouting, bud bursting, seedling, tillering, booting, flowering and maturity stages. Serious cold injury at the bud bursting stage often makes rice seedling rot, inhibits seedling growth and influences the establishment of photosynthetic population, so the cold tolerance at the bud bursting stage (CTB) is considered as one of the most important traits in rice (Han et al, 2004).

Since 1930s, many studies of physiological and genetic mechanism have been made systemically on rice cold tolerance and lots of achievements have been acquired, especially for CTB (Nakaye, 1933; Hirosi, 1940). Yan et al (1999) reported that a continuous distribution with two peaks was observed in a double haploid population (DH) derived from a cross of Nanjing 11/Balilla, when the cold tolerance was assessed by died seedling percentage. They considered that CTB was a quantitative trait controlled by a major gene. However, another result of QTL analysis with simple sequence repeat (SSR) markers for CTB showed that the survival seedling rate was a continuous distribution similar to normal distribution in an F1 population with 200 lines derived from a cross between Milyang 23 and Jileng 1, and they thought that CTB was a quantitative trait controlled by polygenes (Qiao et al, 2005).

MATERIALS AND METHODS

Rice materials

F1 plants derived from a cross between a japonica rice variety Nipponbare and an indica rice variety 9311 were continuously backcrossed to the recipient 9311 to produce BC1F1. Then, 1500 BC1F1 plants were detected with 132 polymorphic SSR markers distributed over the whole rice genome and 139 individuals carrying heterozygous chromosome segments of Nipponbare were selected. These plants were selfed to produce BC4F2 populations. Finally, a total of 95 CSSLs
were obtained from 6192 BC1F2 individuals by molecular marker-assisted selection (MAS). The substitution segments in CSSLs covered most of 12 chromosomes and each CSSL contained a single defined chromosome segment from Nipponbare (Zhu et al, 2009).

Determination of the length of substituted segments in CSSLs

The length of substituted segments in CSSLs was estimated following the method as described by Young and Tanksley (1989). The likelihood of a double crossovers was not inferred in this study, so the chromosome segment flanked by two markers of donor genotype (DD) was considered as 100% donor genotype and the chromosome segment flanked by two markers of recipient genotype (RR) was considered as 0% donor genotype, and the chromosome segment flanked by one marker of donor type and one marker of recipient type (DR) was considered as 50% donor type. The length of DD plus that of two halves of DR was estimated to be the length of a substituted segment.

Evaluation of cold tolerance at the bud bursting stage

The methods of evaluation for CTB were performed as described by Li (1981). Seeds of CSSLs and parents were dried at 45ºC in an air oven for 48 h, then 150 seeds of each line and parent were laid on the filter paper in Petri dish (9 cm) with water and incubated at 30ºC to accelerate germination in an air incubator. When the length of plumule was 5 mm or so, the temperature in the air incubator was adjusted to 5ºC. After low temperature treatment for 10 days, the temperature in the air incubator was adjusted to 30ºC again to recover growth. Then, the survival seedling rate was investigated after 10 days [CTB(%) = Number of survival seedlings/Total number of germinated seeds×100%]. Three duplications were conducted, and means of the duplications were used in data analysis.

QTL analysis of CTB

QTL analysis was adopted from the method described by Liu et al (2004). QTL was detected based on t-test of the difference between the mean of each CSSL and 9311, and a probability level of 0.001 was used as the threshold for detecting a putative QTL. QTL location was determined with the method of substitution mapping (Paterson et al, 1990). If a QTL was identified in different CSSLs with overlapping segment, it would be considered as the same locus in overlapping region; if a QTL was identified in one CSSL and not detected in another one with overlapping segment, it would be located in non-overlapping region of the CSSL detected. The QTL nomenclature followed that of McCouch et al (1997).

RESULTS

Analysis of cold tolerance at the bud bursting stage for parents and CSSLs

A significant difference of CTB was observed in Nipponbare and 9311 and the survival seedling rates of them were 92% and 15%, respectively (Fig. 1). The results indicated that the japonica rice Nipponbare had obviously stronger effect on CTB than the indica rice 9311. In 95 CSSLs, there were 62, 16, 10 and 7 lines with the survival seedling rate below 10%, of 10%–20% and 20%–30% and above 30%, accounting for 65.3%, 16.8%, 10.5% and 7.4% of the total, respectively. The survival seedling rates of CSSL-71, CSSL-75, CSSL-76, CSSL-80, CSSL-122 and CSSL-123 were 38%, 35%, 37%, 56%, 39% and 40% respectively, which were significantly higher than that of 9311. However, according to the standards to evaluate cold tolerance at the bud bursting stage, only CSSL-80 was classified in moderate tolerance and other lines were graded in weak tolerance (Zhang et al, 1996).

Substitution mapping of QTLs for CTB

The results of substitution mapping for CTB showed that a total of four corresponding QTLs in six CSSLs were detected, and qCTB-5-1, qCTB-5-2 and qCTB-5-3 were mapped in the region of RM267–RM1237, RM2422–RM6054 and RM3321–RM1054, which were 21.3 cM, 27.4 cM and 12.7 cM in genetic distance on rice chromosome 5, respectively. qCTB-7 was mapped in the region of RM11–RM2752, which was 6.8 cM in

Fig. 1. Phenotypes at 10 days after recovery from cold stress for parents and some chromosomal segment substitution lines.
DISCUSSION

Many studies confirmed that cold tolerance was a quantitative trait controlled by polygenes in rice. In recent years, several different QTLs for cold tolerance at the bud bursting stage have also been identified on chromosomes 2, 3, 4, 5, 7 and 11 (Teng et al., 2001; Tankeuchi, 2001; Jeong, 2001; Zhan et al., 2003; Fujino et al., 2004). Zhang et al. (2005) demonstrated that a major QTL (qSCT-11), accounting for 30% of the phenotypic variation for CTB on chromosome 11 was linked tightly with a cluster of five tandemly arranged genes. These genes encoding dehydrins might be associated with CTB. Yan et al. (1999) identified another major QTL (Cts7) on chromosome 7, which could explain 39% of the phenotypic variation for CTB. These studies showed that CTB was also a complex quantitative trait controlled by polygenes, and the accuracy of QTL mapping for this trait was easily affected by dissimilar genetic populations, methods for phenotypic identification and environmental conditions.

CSSLs populations could eliminate the interference caused by other genetic background, so it was a very useful material to detect QTLs with minor effects in particular. In this study, six CSSLs had slightly stronger effect of CTB than 9311. It was also proved that QTL analysis with CSSLs could reflect the real effect of quantitative trait loci and improve the detection efficiency of QTLs with minor effects.

A remarkable distinction of cold tolerance was observed between japonica and indica rice. Generally speaking, the cold tolerance of japonica rice was stronger than that of indica rice, and QTLs with positive effect mostly came from japonica rice (Yan et al., 1999; Qiao et al., 2005; Zhang et al., 2005; Zhang et al., 2007; Yang et al., 2009). In this study, the QTLs identified by substitution mapping were all derived from the japonica rice Nipponbare, and it was consistent with previous reports. Among these QTLs, qCTB-5-1 was closely linked with qCTB-5-2 on chromosome 5, so whether they were the same locus is doubtful. Therefore, it was very necessary to add more polymorphic markers in this region to elucidate this problem. Zhang et al. (2007) mapped two QTLs for CTB on chromosome 5 by using a recombinant inbred line (RIL) derived from the cross between a japonica rice Asominori and an indica rice IR24. Comparative analysis of DNA marker on the integrated genetic linkage map (www.gramine.org) indicated that the segments of qCTBP-5-1 from IR24 and qCTBP-5-2 from Asominori were overlapped with those of qCTB-5-2 and qCTB-5-3 in this study, respectively. It was obvious that qCTB-5-2 derived from Nipponbare was a relatively stable and independent locus with strong cold tolerance, and it might be isolated by construction of the secondary population.

The QTL of qCTB-7 detected in the region of RM11–RM2752 was not identical with other QTLs on chromosome 7.
reported in previous studies (Yan et al, 1999; Zhang et al, 2005; Qiao et al, 2005; Yang et al, 2009). Because CTB was a complex quantitative trait controlled by polygenes and the accuracy of QTL mapping could be influenced by many factors including population structure and size, density of genetic markers and evaluation methods, it still needed more experimental evidences to elucidate that this QTL was a new locus for CTB. Fine mapping of these QTLs by construction of the secondary population in further study could provide an important basis for improving the cold tolerance at bud bursting stage in indica rice through pyramiding and recombining QTLs of strong cold tolerance with molecular marker-assisted selection.

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REFERENCES


Li T G 1981. A indoor method of screening cold tolerant rice including population structure and size, density of genetic accuracy of QTL mapping could be influenced by many factors complex quantitative trait controlled by polygenes and the of strong cold tolerance with molecular marker-assisted stage in indica rice through pyramiding and recombining QTLs important basis for improving the cold tolerance at bud bursting period in rice. Sci Agric Sin, 38(2): 217–221. (in Chinese with English abstract)


