QTL Analysis of Anoxic Tolerance at Seedling Stage in Rice

WANG Yang1,2, GUO Yuan1, HONG De-lin1
(1State Key Laboratory of Crop Genetics and Germplasm Enhancement, Nanjing Agricultural University, Nanjing 210095, China; 2Department of Agricultural Resource and Environment, Heilongjiang University, Harbin 150008, China)

Abstract: Coleoptile lengths (CL) of 7-day-old seedlings under anoxic stress and normal conditions were investigated in two permanently segregated populations and their parents in rice (Oryza sativa L.). Using anoxic response index, a ratio of CL under anoxic stress to CL under normal conditions, as an indicator of seedling anoxic tolerance (SAT), QTLs for SAT were detected. Two loci controlling SAT, designated as qSAT-2-R and qSAT-7-R, were detected in a recombinant inbred line (RIL) population (247 lines) derived from a cross between Xiushui 79 (japonica cultivar) and C Bao (japonica restorer line). qSAT-2-R, explaining 8.7% of the phenotype variation, was tightly linked with the SSR marker RM525. qSAT-7-R, explaining 9.8% of phenotype variation, was tightly linked with the marker RM418. The positive alleles of the two loci came from C Bao. Six loci controlling SAT, designated as qSAT-2-B, qSAT-3-B, qSAT-5-B, qSAT-8-B, qSAT-9-B and qSAT-12-B, were detected in a backcross inbred line (BIL) population (98 lines) derived from a backcross of Nipponbare (japonica)/Kasalath (indica)/Nipponbare (japonica). The positive alleles of qSAT-2-B, qSAT-3-B and qSAT-9-B, which explained 16.2%, 11.4% and 9.5% of the phenotype variation, respectively, came from Nipponbare. Besides, the positive alleles of qSAT-5-B, qSAT-8-B and qSAT-12-B, which explained 7.3%, 5.8% and 14.0% of the phenotype variation, respectively, were from Kasalath.

Key words: rice (Oryza sativa); seedling; anoxic tolerance; quantitative trait locus; recombinant inbred line; backcross inbred line

Direct seeding in rice (Oryza sativa L.) is a labor-saving cultivation pattern, and is now becoming more and more popular. Direct seeding is not only labor-saving and highly efficient (Chen, 2003), but also increases number of panicles per unit area and 1000-grain weight (Jing et al, 2008), compared with the traditional transplanting cultivation pattern. In the rice production through direct seeding, one of the key factors affecting grain yield is the seedling establishment in paddy fields (Wu et al, 2006). In flooded or water-saturated soil, seedling rates lowered due to anoxic stress (Fred et al, 1981). Breeding rice cultivars with anoxic tolerance at the seedling stage would be helpful in increasing seedling rate in paddy fields of direct seeding.

Rice seed can germinate under anoxic condition, but root growth and leaf emergence are hindered. To get O2 for the growth of root and leaf, the coleoptile elongates faster and longer under anoxia than normal conditions (Yamauchi et al, 1993). Coleoptiles of varieties with anoxia tolerance had stronger elongation ability than those without anoxic tolerance (Setter et al,1994; Yamauchi and Biswas, 1997), and coleoptiles of japonica varieties elongated faster and longer than those of indica varieties under anxia stress (Hou et al, 2004). We also found genetic variations in anoxic tolerance of seedlings among japonica varieties (Wang et al, 2009). These genetic variations can be utilized to develop varieties with anoxic resistance in rice breeding.

Using the shoot length of seedlings germinated in water at a depth of 20 cm for 5 days in the dark as an indicator of the anoxic germination (AG), Hou et al (Hou et al, 2004) detected five QTLs located on chromosomes 1, 2, 5, 5 and 7 in a population of recombinant inbred lines (RILs). According to the direction of the additive effects, the positive alleles at the 1oci of qAG-1, qAG-G2 and qAG-7 derived from Kinmaze, while at the loci of qAG-5a and qAG-5b, the DV85 alleles increased AG. However, the RIL population mentioned above was derived from a cross...
of Kinmaze (japonica)/DV85 (indica), and was not a breeding population. Studies on QTL mapping for seedling anoxic tolerance by using a breeding population of japonica rice have not been reported yet. In order to discover more genetic information for breeding rice varieties suitable for direct seeding, we conducted QTL mapping for seedling anoxic tolerance using a population of 247 RILs from the cross of Xiushui 79 (japonica cultivar) and C Bao (japonica restorer line), and a population of 98 lines derived from a backcross of Nipponbare (japonica)/Kasalath (indica)/Nipponbare.

MATERIALS AND METHODS

Rice materials

A population of 247 recombinant inbred lines (RILs) from a cross of Xiushui 79 (japonica cultivar) and C Bao (japonica restorer line) and their parents were used. In 2008, the population of F_{10:11} generation was obtained. Another population of 98 backcross inbred lines (BILs) from a backcross of Nipponbare (japonica)/Kasalath (indica)/Nipponbare and their parents were also used. The BIL population of BC_{1}F_{13} was obtained in 2007. Both populations were developed by the single-seed descent method.

Field planting

In 2007, seeds of the BIL population and their parents were sown in a rice seedling bed in May, and the seedlings were transplanted in June at Jiangpu Experimental Station, Nanjing Agricultural University, China. In 2008, the experiment was conducted in the same way for the seeds of the RIL population and their parents.

Each material was transplanted to one plot in a paddy field. Twelve hills per plot were transplanted at a density of 16.7 cm×20.0 cm, with one seedling per hill. Field managements followed traditional practice. Three thousand seeds were harvested from middle five hills of each plot at 45–50 d after heading (harvesting time was determined according to the panicle size). Seed dormancy was broken by keeping the seeds at 50°C in a drying oven for five days.

Measurement of coleoptile length of seedling germinated under anoxic conditions

Two hundred seeds of each material were surface sterilized by immersion in 0.6% sodium hypochlorite solution for 15 min and rinsed with tap water for three times. Then the seeds were soaked in water for 48 h at 25°C. After that, 20 seeds per line which absorbed enough water were put into a plastic culture tray (modified from seedling trays) with 150 compartments (1.9 cm×1.9 cm×3.8 cm) with a draining hole (0.6 cm diameter) under the compartment, and germinated in the depth of 5 cm water. Four seeds were placed at the bottom of each compartment. The plastic culture tray was put on the surface of vermiculite to a depth of 5 cm water in a plastic transfer box (37 cm×25 cm×9 cm). The distance between seeds and water surface was 5 cm. The length of the coleoptile of 7-day-old seedlings was measured from the base of coleoptile to the top and accurate to 1 mm.

The mean of the coleoptile length of 10 seedlings was calculated in each line. The germination experiment was conducted in the GXZ-type incubator under the photoperiod of 16 h darkness at 20°C and 8 h light at 30°C with two replications.

Measurement of coleoptile length of seedling germinated under normal condition

Twenty seeds per line which absorbed enough water were put on the surface of two layers of filter paper covering the slope board at 70-degree tilt position in the germination box. Water in the germination box was kept 3 cm high in order to make paper moist without soaking seeds. The other germination conditions and the measurement of coleoptile length of 7-d-old seedlings were the same as those under anoxic conditions.

Calculation of seedling anoxic response index

The ratio of coleoptile length under anoxic conditions over that under normal conditions was calculated as the anoxic response index of seedling, and used as an indicator of seedling anoxic tolerance.

Data analysis

A genetic linkage map of SSR markers for the RIL
population has been constructed by our laboratory (Guo et al, 2010). The linkage map spans 744.6 cM of rice genome with 74 SSR markers from all 17 individual linkage groups.

The molecular data of the BIL population were obtained from Dr. Yano at the National Institute of Agrobiological Resources, Japan. The 245 RFLP markers were distributed evenly on 12 chromosomes, covering 1179.9 cM of rice genome with an average distance of 4.8 cM between markers.

The mean of the coleoptile length of each line over two replications was used as a unit for the following statistical analysis. QTL detection was carried out by the Composite Interval Mapping (CIM) method in the Win QTL Cartographer 2.5 software (Wang et al, 2006). The LOD scores were calculated every 2 cM on 12 chromosomes, with the threshold of 2.5. A putative QTL was determined between the markers when the LOD score was larger than the threshold. QTL nomenclature followed the propositions of the Rice Genetics Cooperative (McCouch et al, 2007). In order to distinguish QTLs detected in two different populations, ‘R’ was added to the name of QTLs detected in the RIL population, and ‘B’ was added to the name of QTLs detected in the BIL population.

The statistical analyses were executed with the Excel software according to the Method of Experimental Statistics (Gai, 2000).

RESULTS

Difference between two parents and variations in two populations in seedling anoxic response index

In the RIL population, the mean of the seedling anoxic response index of C Bao (9.30±0.20) was significantly higher than that of Xiushui 79 (7.93±0.04) (t=9.63), and the average value of the RIL population was 9.13±1.89 with a range of 4.21–14.41. The seedling anoxic response index of RIL population showed approximately normal distribution and transgressive segregation with a coefficient of variation of 20.7% (Fig. 1-A).

In the BIL population, the mean of the seedling anoxic response index of Nipponbare (4.58±0.45) was significantly higher than that of Kasalath (2.33±0.07) (t=7.00), and the average value of the BIL population was 3.89±0.74 with a range of 2.36–5.56. The seedling anoxic response index of BIL population showed approximately normal distribution and transgressive segregation with a coefficient of variation of 19.1% (Fig. 1-B).

QTLs for seedling anoxic tolerance detected in the two populations

In the RIL population, two QTLs conditioning seedling anoxic tolerance were detected on chromosomes 2 and 7 (Table 1 and Fig. 2). qSAT-2-R explained 8.7% of the phenotypic variances and its positive allele came from C Bao. The amplification product of SSR marker RM525, closely linked to qSAT-2-R, showed a band of 140 bp using the total DNA of C Bao as template. qSAT-7-R explained 9.8% of the phenotypic variances and its positive allele came from C Bao. The amplification product of SSR marker RM525, closely linked to qSAT-7-R, showed a band of 140 bp using the total DNA of C Bao as template.

In the BIL population, six QTLs governing seedling anoxic tolerance were detected on chromosomes 2, 3, 5, 8, 9 and 12 (Table 1 and Fig. 3). These QTLs explained 5.8% to 16.2% of the phenotypic variances. Nipponbare carried positive alleles on the loci of qSAT-2-B, qSAT-3-B and qSAT-9-B. Kasalath possessed positive alleles on the loci of qSAT-5-B, qSAT-8-B and qSAT-12-B.

Fig. 1. Frequency distributions of anoxic response index in rice seedlings of Xiushui 79/C Bao RIL population (A) and Nipponbare/Kasalath/Nipponbare BIL (B) population. Anoxic response index is the ratio of coleoptile length with seed germinated in water at a depth of 5 cm to coleoptile length under normal germination conditions.
Rice Science, Vol. 17, No. 3, 2010

Difference between two parents and variations in the two populations in coleoptile length of seedlings germinated under normal condition

In the RIL population, the average coleoptile length of CBao (5.5±0.1 mm) was significantly higher than that of Xiushui 79 (4.9±0.1 mm) (t=8.40), and the average value of the RIL population was 5.1±0.6 mm with a range of 3.9–7.1 mm. The coleoptile length showed approximately normal distribution and transgressive segregation. The coefficient of variation of the RIL population was 12.5% for the trait (Fig. 4-A).

In the BIL population, the average coleoptile length of Kasalath (11.0±1.0 mm) was significantly higher than that of Nipponbare (5.5±0.3 mm), and the average value of the BIL population was 6.4±1.3 mm with a range of 3.0–10.1 mm. The coleoptile length in the BIL population showed approximately normal distribution and transgressive segregation. The coefficient of variation of the BIL population was 20.2% (Fig. 4-B).

QTLs for seedling coleoptile length detected in the two populations under normal conditions

In the RIL population, only one QTL conditioning coleoptile length was detected. The QTL located at RM525–RM2127 interval on chromosome 2. qCL-2-R explained 5.2% of the phenotypic variances (Table 2 and Fig. 2) and its positive allele came from Xiushui 79.

In the BIL population, four QTLs controlling coleoptile length were detected and located on chromosomes 1, 2, 8, and 11. These QTLs explained 4.0% to 10.3% of the phenotypic variances (Table 2 and Fig. 3). Positive allele of qCL-1-B was derived from Nipponbare.

DISCUSSION

In both of the two populations, QTL controlling seedling anoxic tolerance was detected on chromosome 2. In RIL population, the SSR marker tightly linked to qSAT-2-R was RM525, which located at the position of 118.1 cM from the end of the short arm of chromosome 2 (McCouch et al, 2002). In BIL population, the RFLP marker tightly linked to...
qSAT-2-B was C747, corresponding to SSR marker RM1367 which located at the position of 110.9 cM from the end of the short arm of chromosome 2 (McCouch et al., 2002). Physical distance between qSAT-2-R and qSAT-2-B was 1230 kb according to the physical map (http://rgp.dna.affrc.go.jp/E/IRGSP/download.html). It suggests that qSAT-2-B and qSAT-2-R might be the same QTL controlling seedling anoxic tolerance.

Under normal germination conditions, the

---

**Table 2. QTLs identified for coleoptile length under normal germination conditions.**

<table>
<thead>
<tr>
<th>Population and QTL</th>
<th>Marker interval</th>
<th>Distance (cM)</th>
<th>LOD</th>
<th>Variance explained (%)</th>
<th>Additive effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>RIL population</td>
<td>qCL-2-R</td>
<td>RM525–RM2127</td>
<td>13.0</td>
<td>2.50</td>
<td>5.2</td>
</tr>
<tr>
<td></td>
<td>qCL-1-B</td>
<td>C1370–C1225</td>
<td>2.0</td>
<td>4.12</td>
<td>10.3</td>
</tr>
<tr>
<td></td>
<td>qCL-2-B</td>
<td>C1221–G275</td>
<td>0.9</td>
<td>2.52</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>qCL-8-B</td>
<td>C1121–R902</td>
<td>0.0</td>
<td>3.18</td>
<td>8.1</td>
</tr>
<tr>
<td></td>
<td>qCL-11-B</td>
<td>C1172–S2260</td>
<td>1.2</td>
<td>6.39</td>
<td>9.7</td>
</tr>
</tbody>
</table>

*Bold letters indicate the nearest marker. *aDistance from the nearest marker to putative QTL. *b+'means that positive alleles come from Xiushui 79 in the RIL population or from Nipponbare in the BIL population, respectively; '-' means that positive alleles come from C Bao in the RIL population or from Kasalath in the BIL population, respectively.*

---

**Fig. 3. Chromosomal location of QTLs for seedling anoxic tolerance and for coleoptile length under normal germination conditions in the BIL population.**
difference of coleoptile length between Xiushui 79 and C Bao was only 1 mm and the coefficient of variation was 12.5% in the RIL population. However, the anoxic response index of seedling in the two parents was significantly different. The average coleoptile length of seedlings of the RIL population (247 lines) under the anoxic stress was nine times as that under the normal condition. In addition, the coefficient of variation of seedling anoxic response index also increased. These results indicate that under the anoxic stress, the differences of the elongation ability of seedling coleoptile became larger among the population lines and the two parents. Furthermore, as the difference of seedling coleoptile length under the normal condition was small among the population lines and between parents, the anoxic response index was more suitable to reflect the anoxic tolerance among lines. Therefore, QTLs controlling seedling anoxic tolerance detected in the RIL population derived from japonica-japonica cross were more reliable than those detected in the BIL population derived from indica-japonica cross. Since the RIL population had smaller difference in seedling coleoptile length among lines than the BIL population under the normal conditions, thus the variation of the anoxic response index directly reflecting the variation of seedling coleoptile length under the anoxic conditions.

In the BIL population, five QTLs for seedling anoxic tolerance did not overlapped with QTLs for seedling coleoptile length germinated under the normal conditions except the locus \( qSAT-8-B \).

\( qSAT-8-B \) and \( qCL-8-B \) were both located between R1813 and C1121 on chromosome 8, with a genetic distance of 1.0 cM. Genes controlling coleoptile elongation under anoxic stress were different from those under normal conditions. In the anoxic stress, anaerobic carbohydrate catabolism takes the place of aerobic carbohydrate catabolism, and sugars appear to play a signaling role under anoxia, with several genes such as \( RAMY3D, PDC, ADH1 \) and \( ADH2 \) indirectly up-regulated by anoxia-driven sugar starvation (Gibbs et al, 2000). In the anoxic rice coleoptile, six among 34 cell expansion genes were up-regulated (Rasika et al, 2007), especially \( EXP \) and \( EXPB12 \). Furthermore, the expressions of some genes encoding functional proteins altered under anoxia. For example, rice \( HSP20 \) showed a higher expression, while some dehydrogenase genes were down-regulated under anoxia. Therefore, genes controlling coleoptile elongation under anoxic stress were different from those in air.

**REFERENCES**


McCouch S R, Teytelman L, Xu Y. 2002. Development and


