QTLs for Seedling Growth of Direct Seeded Rice under Submerged and Low Temperature Conditions

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Abstract: Quantitative trait loci (QTL) affecting the germination rate, coleoptile length, and shoot dry weight were analyzed under submerged and low temperature conditions using inbred lines derived from crossing the rice cultivars Ouu 365 and Arroz da Terra. The QTLs that increased the germination rate and shoot dry weight by Arroz da Terra allele were detected on the same region of chromosome 3, where the low temperature germinability gene, qLTG3-1, localized, suggesting that greater germinability might lead to increased shoot growth in paddy fields. The QTLs that increased the coleoptile length by Ouu 365 and Arroz da Terra alleles were detected on chromosome 8 and 11, respectively. The analysis of the known germinability genes suggested that functional allele of qLTG3-1 increased germination rates of the inbred lines. However, Rc which induced red pigmentation in pericarp declined the germination rates of the lines with functional qLTG3-1.

Key words: Coleoptile, Germination, Quantitative trait locus, QTL, Rice, Oryza sativa L, Seedling vigor.

Direct seeding of rice (Oryza sativa L.) in submerged paddy fields saves labor and cost compared with transplanting. However, submerged and low-temperature conditions in the sowing season inhibit seedling growth. Especially in the northern part of Japan, cool temperatures in spring often inhibit the seedling growth of direct seeded rice. Strong seedling vigor, germinability and early seedling growth, are considered essential factors for direct seeding even in submerged and low temperature conditions because delayed emergence of seedlings results in increased seedling mortality (Jones and Peterson, 1976; Yamauchi and Winn, 1996; Ogiwara and Terashima, 2001).

Marker-assisted selection is a powerful tool for breeding cultivars that have strong seedling vigor, which is easily affected by the environment and is difficult to evaluate and select. Quantitative trait loci (QTL) analysis and identification of genes affecting seedling vigor are necessary to identify genetic markers. QTLs affecting seedling vigor, low temperature germinability (Suh et al., 1999; Miura et al., 2001; Fujino et al., 2004; Zhang et al., 2005a, 2005b; Zhou et al., 2007; Abe et al., 2012; Yano et al., 2012), and seedling emergence under field conditions (Zhou et al., 2007; Iwata et al., 2010) have been reported. These studies suggested that seedling vigor is affected by many genetic and environmental factors.

In this study, we identified QTLs that increase seedling vigor even in the cool and submerged paddy fields in the Tohoku region in the northern part of Japan. We used a set of backcrossed inbred lines (BILs) produced with Arroz da Terra as the donor parent and Ouu 365 as the recipient parent. Arroz da Terra is known to have strong seedling vigor even under cool and submerged conditions (Ogiwara and Terashima, 2001; Iwata et al., 2010). QTLs for the shoot dry weight in the submerged paddy fields in the Tohoku region were detected in the BILs. We also detected the seedling vigor-related traits, germinability and coleoptile length, under controlled temperature conditions and compared them with the QTLs for the shoot dry weight detected in field conditions. Additionally, we analyzed the known germinability genes, qLTG3-1 and Rc, of BILs. Previously, Fujino and Iwata (2011) showed...
that Arroz da Terra carries a functional allele of qLTG3-1, which increases the low temperature germinability (Fujino et al., 2004). Arroz da Terra also harbors another germinability gene, Rc, which causes red pigmentation in pericarp and has a pleiotropic effect to promote biosynthesis of abscisic acid resulting in the enhancement of seed dormancy (Gu, et al., 2011). On the other hand, Ouu 365 has neither the functional alleles of qLTG3-1 nor Rc. In this study, we performed comprehensive analysis of the interaction of the two genes in the germinability of rice seeds using the same BILs.

Materials and Methods

1. Seed materials

The F1 plants between Ouu 365 and Arroz da Terra were backcrossed with Ouu365. Then, 104 BC1F9 plants were developed by the single seed descent method. Seeds from all of the BC1F9 plants (BILs) and the parent cultivars were harvested from the experimental paddy fields in Daisen campus of NARO Tohoku Agricultural Research Center (Daisen, Akita, Japan, N39.29, E140.29) in 2007. The harvested seeds were stored at 6°C for 7 – 8 months until they were used for the experiments.

2. Analysis of the germination rate

For the analysis of germination rate, 50 seeds per line were sterilized for 1hr in 50-fold diluted antiformin containing 100 μL L⁻¹ Triton X-100. After initial sterilization, seeds were rinsed with water 3 times and imbibed in 3.5 cm deep water in a polycarbonate pot (70 mm in diameter, 51 mm in length, cover 70 mm in length with a vent hole; “Agripot”, Iwaki Glass Co. Tokyo, Japan) at 16°C in the dark in an incubator. The number of the germinated seeds was recorded every day for 10 days after the start of imbibition. Experiments were replicated 3 times. The mean value of the germination rates after 4 days of imbibition in 3 replications was used for the QTL analysis.

3. Analysis of the coleoptile length

Seeds were sterilized and germinated as described above. One germinated seed was placed in a test tube (10 mm diameter, 100 mm long) with 8.5 cm water depth so that coleoptiles did not reach the water surface, and stop the elongation. Fifteen tubes were packed in a polycarbonate pot (Agripot; described above) and incubated at 16°C in the dark for 7 days in an incubator, and then the coleoptile lengths of 45 plants per line were measured. The mean value of the coleoptile length of 45 plants was used for the QTL analysis.

4. Analysis of the seedling growth in a submerged field

Gray lowland soil was obtained from a paddy field located in Daisen campus of NARO Tohoku Agricultural Research Center. Sieved soil was filled in nursery boxes for seedling mat of 580 mm × 280 mm × 28 mm (length × width × depth) to 10 mm below the top edge of the boxes. Seeds were sterilized and rinsed as described above and then soaked in water for 24 hr at room temperature. Fifty seeds per line were sown on the surface of the soil linearly in the nursery boxes. Eight lines per nursery box were sown and covered with commercial nursery soil at a depth of 8 mm. Then, nursery boxes were placed in the continuously irrigated paddy field in Daisen campus of NARO Tohoku Agricultural Research Center. The depth from the seeds to the water surface was maintained around 3 cm. At 35 days after the sowing, the seedlings that reached the second leaf stage were regarded as the established seedlings, and the percentage of the established seedlings was determined. All established seedlings were sampled, removed the roots and seeds, dried at 80°C for 3 days and shoot dry weight was measured. Experiments were replicated 3 times, and 50 seeds per line, one repeat per line, were used in one replication. The first replication started on 7 May, the second replication started on 8 May, and the third replication started on 9 May in 2008. During the field experiments, the mean values of the air and soil temperatures were 15.6°C and 17.6°C, respectively. The mean values of the percentage of established seedlings in the three replications were used for the QTL analysis. Shoot dry weights per plant were determined in each replication, and the mean values of the 3 replications were used for the QTL analysis.

5. QTL analysis

Total DNA was extracted from the young leaves at the vegetative stage of parental cultivars and BC1F9 plants (BILs), from which the seeds for in the analysis of seedling vigor were harvested, by the cetyl-trimethyl-ammonium bromide (CTAB) method (Murray and Thompson, 1980). For genotype analysis, 124 simple sequence repeat (SSR) markers that had polymorphisms between the parental cultivars were examined in all of the BILs. Linkage analysis was performed using MAPMAKER/EXP 3.0 (Lander et al., 1987), and the QTLs were determined with QTL Cartographer 2.5 (Wang et al., 2010). The threshold to detect QTLs was determined by 1000 permutation tests at a probability level of 0.05.

6. Genotype analysis of qLTG3-1 and Rc

Total DNA was extracted from the BILs and parental cultivars as described above. For the genotype analysis of qLTG3-I gene, the region including the 71-bp deletion in the open reading frame of qLTG3-I was amplified by PCR with the S103ap primers (Fujino et al., 2008). The genotype of Rc gene of the BILs were visually determined by pericarp colors, as red or white.
Results

1. Phenotypic variation for seedling vigor-related traits

The mean germination rates of Arroz da Terra and Ouu 365 after 4 days of imbibition were 26.7% and 39.3%, respectively, which were not significantly different (Fig. 1A), and that of BILs ranged from 0% to 93.3% (Fig. 2A). The mean germination rates of Arroz da Terra and Ouu 365 after 10 days of imbibition were 99.3% and 97.3%, respectively, which were not significantly different (Fig. 1A), and that of BILs ranged from 91.3% to 100%. The coleoptile length of Arroz da Terra (28.1 mm) at 7 days after germination was significantly larger than that of Ouu 365 (24.8 mm) (Fig. 1B), and that of BILs ranged from 18.3 to 31.8 mm (Fig. 2B). The shoot dry weight plant$^{-1}$ of Arroz da Terra (13.2 mg plant$^{-1}$) at 35 days after seeding in the paddy fields was significantly heavier than that of Ouu 365 (7.7 mg plant$^{-1}$) (Fig. 1C), and that of BILs ranged from 4.3 to 13.0 mg (Fig. 2C). The mean seedling establishment rates in the paddy fields of Arroz da Terra and Ouu 365 were 55.3% and 54.0%, respectively, which were not significantly different (Fig. 1D), and that of BILs ranged from 24.7% to 64.7% (Fig. 2D). In the BILs, the germination rate after 4 days of imbibition, coleoptile length at 7 days after germination, and shoot dry weight plant$^{-1}$ in the paddy fields were significantly and positively correlated (Fig. 3).

2. QTLs for seedling vigor-related traits

One QTL that increased the germination rate after 4 days of imbibition by Arroz da Terra allele was detected on chromosome 3 (Table 1 and Fig. 4). Two QTLs for the coleoptile length at 7 days after germination were detected on chromosome 8 and 11. Among them, the QTL on chromosome 8 increased coleoptile length by Ouu 365 allele, while the QTL on chromosome 11 increased coleoptile length by Arroz da Terra allele. One QTL, which increased shoot dry weight plant$^{-1}$ in the paddy fields, was detected on chromosome 3, which was the same region as the putative QTL for the germination rate after 4 days of imbibition by Arroz da Terra allele.
imbibition. No significant QTL for the seedling establishment rates was detected in the paddy fields.

3. Germination rates and genotypes of \( qLTG3-I \) and \( Rc \)

PCR analysis showed that Ouu 365 had a deletion in the open reading frame of \( qLTG3-I \). The germination rate after 4 days of imbibition in genotypes of \( qLTG3-I \) and \( Rc \) of BILs are presented in Fig. 5. Analysis of variance showed that the germination rate was significantly higher in the lines that had functional \( qLTG3-I \) than those that had a nonfunctional allele (Fig. 5B). We could not detect any significant effect of the \( Rc \) among the lines that carries nonfunctional allele of \( qLTG3-I \). On the other hand, the line with the \( Rc \) showed a lower germination rate than those without \( Rc \) among the lines that have functional allele of \( qLTG3-I \).

Discussion

QTLs that increased the germination rate and shoot dry weight by Arroz da Terra allele were detected in the same region on chromosome 3 (Table 1 and Fig. 4), suggesting that the chromosomal region of Arroz da Terra accelerated the germination, and the faster germination by this allele resulted in increased shoot dry weight. Additionally, the

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significantly correlated with the germination rate. However, \( R_c \) that induces the red pericarp color decreased the germination rate of the lines with functional \( qLTG3-1 \) (Fig. 5B). This may be why Arroz da Terra did not exhibit a significantly higher germination rate than Ouu 365 (Fig. 1A), although it had a functional \( qLTG3-1 \). Arroz da Terra has the \( R_c \) gene, which is known to enhance the seed 

germination rate of BILs had a positive correlation with the shoot dry weight (Fig. 3B). This result also suggested that greater germinability improved the seedling growth in the paddy field conditions. Introduction of QTLs that increase the germination rate might contribute to production in the cultivars that grow faster in the paddy fields. On the other hand, Arroz da Terra had a lower germination rate than Ouu 365, though the shoot dry weight of Arroz da Terra was significantly higher than that of Ouu 365 (Fig. 1). These results indicated that shoot growth was affected not only by the germinability but also by some other factors. In BILs, coleoptile length and shoot dry weight were positively correlated (Fig. 3D), suggesting that the higher coleoptile growth rate contributed to the increase in shoot dry weight, though the effect of coleoptile elongation might be too weak to detect in the QTL analysis for shoot dry weight. The seedling growth might be determined by several QTLs.

The low temperature germinability gene, \( qLTG3-1 \), localized near the Lod peaks of the QTLs for the germination rate and shoot dry weight (Table 1 and Fig. 4). The functional \( qLTG3-1 \) was found in Arroz da Terra (Fujino and Iwata, 2011). On the other hand, Ouu 365 had a deletion in the open reading frame of \( qLTG3-1 \), which induced loss of the function. Genotype analysis of \( qLTG3-1 \) in BILs showed that functional \( qLTG3-1 \) increased the germination rates (Fig. 5B). Therefore, the functional \( qLTG3-1 \) from Arroz da Terra might be essential for the QTL to increase the germination rate. The functional \( qLTG3-1 \) might also be essential for the QTL to increase shoot dry weight, since the shoot dry weight of BILs was significantly correlated with the germination rate. However, \( R_c \) that induces the red pericarp color decreased the germination rate of the lines with functional \( qLTG3-1 \) (Fig. 5B). This may be why Arroz da Terra did not exhibit a significantly higher germination rate than Ouu 365 (Fig. 1A), although it had a functional \( qLTG3-1 \). Arroz da Terra has the \( R_c \) gene, which is known to enhance the seed
dormancy (Gu et al., 2011). It is necessary to eliminate the allele that has a negative effect and introduce the positive allele to improve germinability. On the other hand, the \(Rc\) allele was not detected in the QTL analysis. In addition, in the lines that had a nonfunctional \(qLTG3-1\), no difference in the germination rate was detected between the \(Rc (+)\) and \(Rc (-)\) lines (Fig. 5B). \(Rc\) does not affect the dormancy in some cultivars, and its effect has been reported to be influenced by the genetic background (Gu et al., 2011). \(Rc\) might be effective in inducing dormancy in the functional \(qLTG3-1\) background, but not in the nonfunctional \(qLTG3-1\) background.

There was a significant difference between Arroz da Terra and Ouu 365 in the coleoptile length (Fig. 1B), but not in the seedling establishment rate in the paddy fields (Fig. 1D). Furthermore, the coleoptile length of BILs was not significantly correlated with the seedling establishment rates (Fig. 3E). By contrast, Ogiwara and Terashima (2001) reported that rapid coleoptile elongation after the germination was necessary to improve the seedling establishment rate. Moreover, the cultivars with a longer coleoptile had a higher coleoptile elongation rate (Furuhata et al., 2007). Miura et al. (2002) reported that the long-coleoptile trait improved the seedling establishment rate in the submerged fields. One reason why the seedling establishment rates were not correlated with the coleoptile length in this study might be that the differences in coleoptile length between the two lines were relatively small. The coleoptile length of Arroz da Terra was about 1.1 times of Ouu 365. The longest coleoptile length was 1.7 times the shortest one in the BILs. Miura et al. (2002) reported that a line that had a coleoptile more than twice as long as that of the control cultivar, had a higher seedling establishment rate.

Recently, non-germinated iron-coated seeds are used for the direct seeding in submerged paddy fields to prevent the floating of seedlings (Yamauchi, 2012). The iron-coated seeds are subjected to seed hardening, soaking in water and re-drying, to improve seedling vigor (Mori et al., 2012; Yamauchi, 2012). If cultivars with a long coleoptile and high germination rate could be bred, the seed hardening process would be unnecessary and this would be labor-saving. Cultivars that are suitable for direct seeding in cool regions, such as Tohoku region, and have a higher growth rate under cool and submerged paddy fields than the conventional cultivars need to be produced by pyramiding the QTLs to increase the germination rate and coleoptile length.

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