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Mapping quantitative trait loci controlling low-temperature germinability in rice

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Abstract : Low-temperature germination is one of the major determinants for stable stand establishment in the rice direct seeding method in temperate regions and at high altitude areas. Quantitative trait loci (QTL) controlling low-temperature germinability in rice were identified using 96 introgression lines (ILs) derived from a cross between *Oryza rufipogon* and the Korean *japonica* cultivar, 'Hwaseongbyeo'. The germination rate at 15℃ was measured to represent low-temperature germination and used for QTL analysis. The germination rate at 15℃ for 7 days of *Oryza rufipogon* and Hwaseongbyeo was 93.3 and 28.7%, respectively, and that of progenies ranged from 0 to 48%. A linkage map was constructed using 135 simple sequence repeat (SSR) markers. Five putative QTLs associated with low-temperature germination were detected on chromosomes 1, 3, 4, 10 and 11. The QTL, *qltg10* on chromosome 10 accounted for 19.2% of the total phenotypic variation for low-temperature germinability. Four additional QTL, accounted for 10.4 - 15.1% of the total phenotypic variation. The *O. rufipogon* alleles in all detected QTLs loci increased the low-temperature germination rate. No QTL associated with low temperature germinability has been detected near the *qltg10* QTL in this study suggesting that *qltg10* is a new QTL. The locus, *qltg10* is of particular interest because of its independence from undesirable height and maturity effects. The DNA markers linked to the QTL for low temperature germinability would be useful in selecting lines with enhanced low temperature germinability in rice breeding program.

Key words **:** Rice, Low temperature germinability, QTL, Introgression lines

I. Introduction

Rice, being a temperature-sensitive crop, experiences yield loss due to low temperature (Mackill, 1995; Peyman and Hashem, 2010). About 15 million hectares of rice fields in 24 different countries is under threat from cold weather (Zeng et al., 2009). In Korea, rice experiences low temperature in northern and high altitude areas. Rice plants growing in these areas are regularly exposed to low temperature during different stages of growth i.e-vegetative, reproductive and ripening. The vegetative phase comprises of different stages namely the germination, seedling and tillering. Occurrence of low temperature stress either at germination or seedling stage affects seed germination and inhibits seedling establishment leading to non-uniform crop maturation (Lou et al., 2007). In this regard, low temperature germinability is one of the major determinants for stable stand establishment in temperate, high altitude areas. In Korea, the direct seeding method has become increasingly important (Suh et al., 1999). Vigorous germination at low temperature is an important character to make stable establishment of seedlings in the direct seeding method.

In spite of the significance of low-temperature germinability in rice cultivation, the genetic mechanism of this trait is not well understood. Low-temperature germinability is considered to be a quantitative trait controlled by several genes as well as environmental factors. The linkage relationships between the genes for low-temperature germination and several morphological marker genes were examined using japonica linkage

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testers (Sasaki et al., 1973).

QTL studies were carried out to identify genes underlying low temperature stress at different developmental stages. These QTL analyses have revealed that low-temperature germination is controlled by several genes (Suh et al., 1999; Miura et al., 2001; Fujino et al., 2004). Three QTLs for low-temperature germination were detected using 98 BC_1F_1 plants from a cross between Milyang23 and Hapcheonaengmi3 (Suh et al., 1999). Five QTLs for low-temperature germination were detected using 98 backcross inbred lines from a cross between Nipponbare and Kasalath (Miura et al., 2001). Three QTLs controlling low-temperature germinability were identified using 122 backcross inbred lines derived from a cross between temperate *japonica* varieties, 'Italica Livorno' and 'Hayamasari' and a major QTL on chromosome 3, qLTG3-1, explained >30% of the total phenotypic variation in the mapping population and was localized to a 3.7-cM interval on chromosome 3 (Fujino et al., 2004). Subsequently, qLTG3-1 was cloned by a map-based strategy, and it was revealed that qLTG3-1 encodes a novel protein of 184 amino acids with unknown function (Fujino et al., 2008).

However, the number of reports on QTL analysis of the genes for the low temperature germinability in the *Oryza rufipogon* is quite rare. This study was conducted to detect QTLs for low temperature germinability using an introgression line population (ILs) derived from a cross between the *Oryza rufipogon* and the Korean *japonica* cultivar Hwaseongbyeo.

II. Materials and methods

1. Plant materials

96 BC_4F_8 lines were employed in this study and the scheme of the population development is described in the paper by Yun et al. (2010). The wild rice *O. rufipogon* (IRGC 105491) was used as a pollen parent in crosses with O. sativa spp. *japonica* cv. Hwaseongbyeo, an elite Korean cultivar, followed by two successive backcrosses made with Hwaseongbyeo as the recurrent parent (RP). Among 820 BC_2F_1 plants, 172 individuals were selected based on phenotypic acceptability and selfed to generate BC_2F_2 families for QTL analysis. For the present study, 98 BC_2F_2 lines, were selected as the basis for NILs development. These lines were backcrossed to Hwaseongbyeo two times and then allowed to self, generating 96 BC_4F_8 lines.

2. Evaluation of low-temperature germinability

The 96 BC_3F_8 lines and the 2 parents were evaluated for germinability at 15℃. The geminability was determined using kernels that had been harvested 30 days after flowering, dried at 45℃ for 2 days. Thirty seeds per line were placed on a filter paper in a 9 cm Petri dish, and 10 mL of distilled water was added. The dishes were placed in an incubator at 15℃. The number of germinated seeds was counted daily in three replications. Data of the germination rate at 7 days after incubation were used for QTL analysis because a large parental difference was observed at 7 days. Also, the germinability of two parents was tested at 30℃, the optimum temperature for germination in rice.

3. DNA extraction and SSR analysis

Fresh leaves were collected from the parents and 96 BC_3F_8 lines and used for DNA extraction. DNA was extracted from the fresh leaves by method as described in Causse et al., (1994). A total of 131 SSR markers polymorphic between Hwaseongbyeo and *O. rufipogon* were surveyed to detect the *O. rufipogon* introgressions in ILs,. The PCR reaction mixture containing 30ng of DNA, 1 unit of Taq polymerase, 2.5 uM each d NTP, 8 uM F, R primer, and 10 x PCR buffer (10 mM Tris -HCl pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.1% Gelatin). Amplification was accomplished with the Thermo (BioRad) by using the step-cycle program set to denature at

94℃ for 5 min, subsequent denaturing was at 94℃ for 1 min., annealing was 55℃ for 1 min., extension was 72℃ for 1 min., and step 2 through 4 were repeated for a total 35 cycles with a final extension at 72℃ for 5 min. PCR products were run on 4% polyacrylamide denaturing gel for 2 hr at 1800-2000V, and marker bands were revealed using the silver staining kit purchased from Bioneer Co., Korea (Panaud et al., 1996, www.bioneer.co.kr). Amplified DNA fragments showing clear polymorphism were used for the analysis of ILs and linkage mapping.

4. QTL analysis

The distance and orientation between markers were based on the previously published map (McCouch et al., 2002). A single point analysis (SPA) was performed to determine the effect of each marker on each trait. In SPA, QTL was declared if the phenotype was associated with a marker locus at $p<0.005$ or with two adjacent marker loci at $p<0.01$. Statistical analysis was done using the software Qgene (Nelson, 1997). The proportion of the total phenotypic variation explained by each QTL was calculated as an R^2 value, from the regressions of each marker/phenotype combination

III. Results

1. Comparison of the parents for germinability

The gemination rate at 30℃ for 3 days after incubation of Hwaseongbyeo and *O. rufipogon* was 100%. No difference between the parents was observed at 30℃. However, a difference between the parents was sown at 15℃ condition (Fig. 1). *O. rufipogon* began germinating at the 3rd day after the start of incubation, and 3 days thereafter almost all of the seeds germinated. In contrast, Hwaseongbyeo began germinating at the 6th after the start of incubation, and 5 more days were needed for near-completion of all germination. The germination rate at 15℃ for 7 days of *O. rufipogon* and Hwaseongbyeo was 93.3 and 21.7%, respectively. The germination rates of ILs varied continuously from 0 to 48.3% at 7 days after incubation (Table 1, Fig. 2).

2. Variation in the ILs

The 96 BC_3F_8 lines and the 2 parents were evaluated for germinability at 15℃. The germination rate at 7 days of *O. rufipogon* and Hwaseongbyeo was 93.3 and 21.7%, respectively. ILs displayed a wide range of varia-

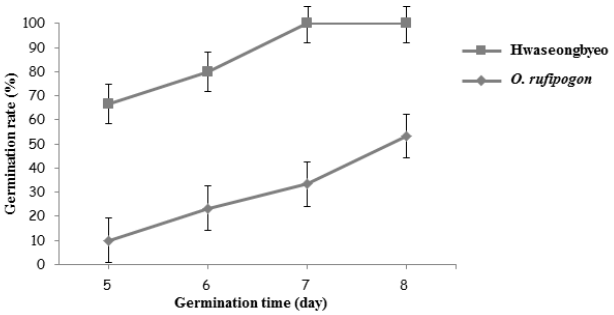

Fig. 1. Changes in the germination rate of the parents, *O. rufipogon* and Hwaseongbyeo at 15℃.
* The circles and the boxes on each day show the means with standard deviation.

Table 1. Comparison of germinability at 15℃ for the parents and ILs at 7 days after incubation

Lines	Germinability (%)
O. rufipogon	93.3 ± 9.4
Hwaseongbyeo	21.7 ± 11.8
96 BC_3F_8	Mean 13.3 ± 12.75 (0 - 48.3)[1)]

[1)] Number in the parenthesis indicates range.

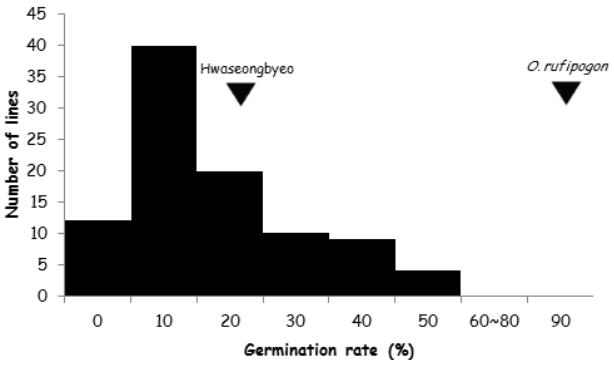

Fig. 2. Frequency distribution of germination rate at 15℃ for ILs at 7 days after the start of incubation.

tion from 0 to 48.3% at 7 days after incubation (Table 1, Fig. 2). The distribution of germinability in the ILs displayed continuous variation and transgressive lines that fell beyond the low mean of the parent were observed. Most of the lines showed similar level of germinability as the recurrent parent, Hwaseongbyeo.

3. QTLs for low-temperature germinability

For parental polymorphism screening with SSR markers, 296 of 460 (64.3%) markers amplified scoreable and reproducible polymorphic bands. The genotypes of 96 ILs were surveyed by 131 markers. Five putative QTLs associated with low-temperature germinability were detected (Fig. 3, Table 2), and the *O. rufipogon* alleles in all detected QTLs loci increased the low-temperature germination rates. The QTL with a large effect, *qltg10*, was mapped at the marker RM147 and RM258 on the chromosome 10 (Fig. 3). The phenotypic variation explained by *qltg10* was 19.2%. Four additional QTLs,

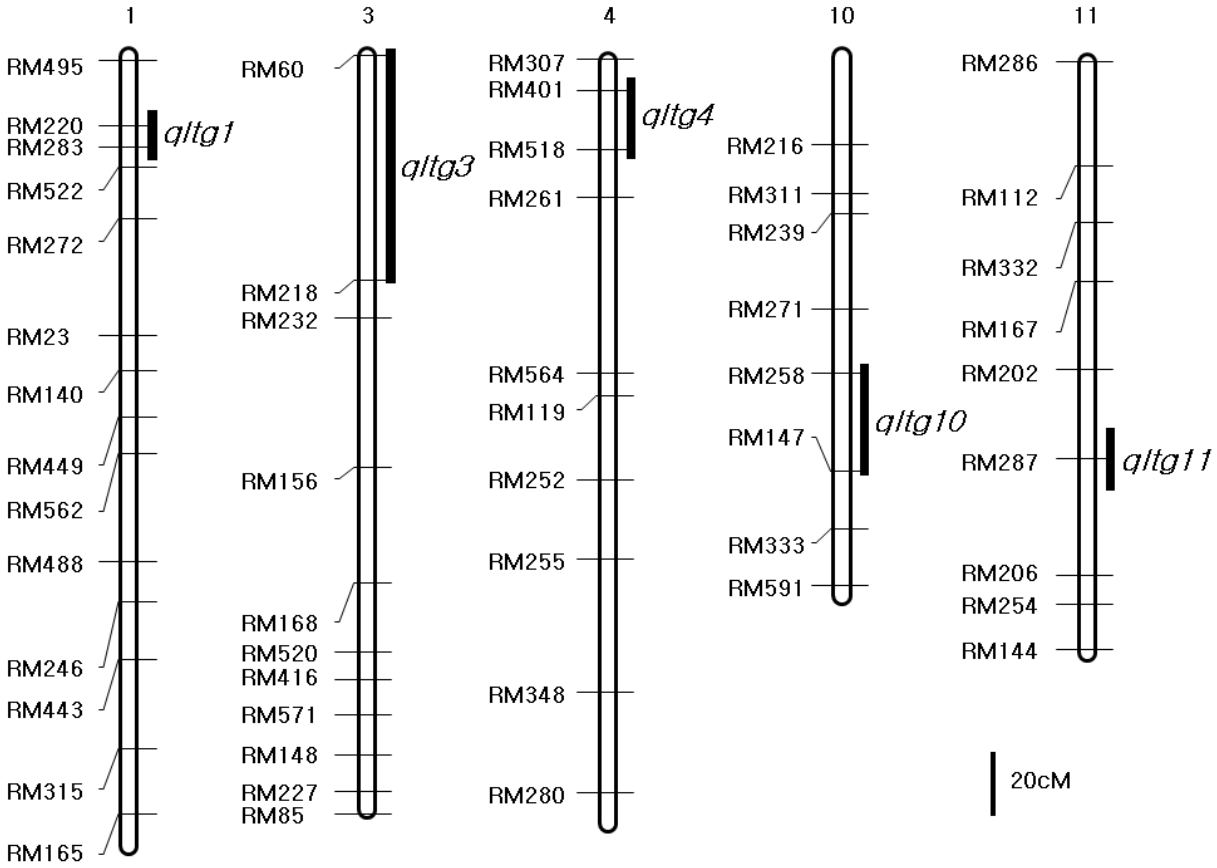

Fig. 3. Chromosomal locations of QTLs for low-temperature germinability in rice.

Table 2. Characteristics of QTLs for low-temperature germinability.

QTL	Chr.	Marker interval	P	LOD	R2	AE[1)]
qltg1	1	RM220-RM283	0.01	2.35	12.1	5.1
qltg3	3	RM60-RM218	0.01	2.13	10.4	4.7
qltg4	4	RM401-RM518	0.0050	2.75	15.1	5.3
qltg10	10	RM258-RM147	0.00001	4.29	19.2	6.4
qltg11	11	RM287	0.0024	2.70	12.6	5.5

[1)]AE(additional effect) = (*O. rufipogon* homozygote - Hwaseongbyeo homozygote)/2

qltg1, *qltg3*, *qltg4*, and *qltg11* on chromosomes 1, 3, 4, and 11 were detected and accounted for 12.1%, 10.7%, 15.1%, and 12.6% of the total phenotypic variation, respectively.

IV. Discussion

Low-temperature germinability is one of the major determinants for stable stand establishment in the direct seeding method in temperate regions and at high altitudes of tropical regions. Previous genetic analyses have revealed that low-temperature germination is controlled by several genes (Sasaki et al., 1973; Miura et al., 2001). However, reports on genetic analysis of the genes for the low temperature germinability in wild species is rare.

In this study, a set of introgression lines carrying wild rice (*O. rufipogon*) segments were developed from a cross between Hwaseongbyeo and *O. rufipogon*. A total 131 SSR markers were used to detect chromosomal segments introgressed from *O. rufipogon* in 96 ILs. 5 QTLs related to low-temperature germinability were detected and the *O. rufipogon* alleles in all detected QTLs loci increased the low-temperature germination rates. Among the 5 QTLs, *qltg10* on chromosome 10 located near the SSR markers, RM258 and RM 147, explained a large part of the total phenotypic variation in the ILs population (19.2%).

The location of the QTLs detected in this study was compared with that of the previous reports. A number of reports (Suh et al., 1999; Miura et al., 2001; Hou et al., 2004) detected QTL for low temperature germinability on chromosome 11. The QTL, *qltg11* detected in this study shared the similar location as those identified in the 3 previous reports and this result suggests the possibility that these QTLs are allelic. Three QTLs for low-temperature germination have been identified by studies of crosses between temperate *japonica* Italica Livorno and Hayamasari (Fujino et al., 2004). One QTL, qLTG-3-1 near the marker GBR3001 was located on chromosome 3, and the Italica Livorno allele in this QTL increased low temperature germination (Fujino et al., 2004). Based on the chromosomal location, this QTL appears to correspond to the *qltg3* detected in this study. Further analysis will be required to prove the allelic relationship between these QTLs. Also, the *qltg4* QTL detected in this study appears to be the same as those identified in the *japonica*/*indica* population (Miura et al., 2001) and *japonica*/*japonica* population (Fujino et al., 2004). However, the allelic relationship among these QTLs needs to be clarified. The other QTLs detected in this study appear to be different from those identified in the previous QTL studies.

So far, no QTL associated with low temperature germinability has been detected near the *qltg10* QTL in this study suggesting that *qltg10* is a new QTL for low temperature germinability. Having identified a new QTL for increased low temperature germinability coming from an accession of *O. rufipogon* not widely utilized in rice breeding programs, the aim of our marker-assisted introgression program was to fix the favorable allele (from *O. rufipogon*) of this QTL in elite cultivars genetic background with as little additional *O. rufipogon* (donor) chromosome as possible. The locus, *qltg10* is of particular interest because of its independence from undesirable height and maturity effects (data not shown). However, additional experiments are needed to determine the effect of *qltg10* on other agronomic traits.

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