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Identification of QTL for Early Heading Date of H143 in Rice

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Abstract

Rice is a facultative short-day plant that flowers in response to reduced day lengths. This study was conducted to identify quantitative trait loci (QTL) for the early heading date (EHD) using H143 line showing extreme EHD compared to other regular cultivars in rice. The *japonica* H143 was crossed with a *japonica* cultivar 'Dongjinbyeo' as well as a tongil cultivar 'Milyang23' to measure the inheritance mode of EHD and identify major QTL conferring EHD, respectively. Pooling test revealed that segregation distortion occurred on chromosome 7 and subsequent linkage map was constructed using 10 SSR markers. QTL analysis using Q-gene 3.06 revealed that the EHD trait in H143 was largely controlled by two major QTL, EH7-1 and EH7-2, accounting for more than 40% of genetic variation that were closely related to the previously reported QTL, *Hd4* and *Hd2*, respectively. This result suggests that these two QTL markers may be a useful source for the control of heading date in rice breeding programs.

Key words: QTL analysis, early heading date (EHD), rice, H143.

Introduction

Heading date is a complex trait controlled by multiple genetic and epigenetic factors. Many environmental factors, such as day length, temperature, light intensity, and nutrients, control heading date in rice, and a number of genes participating in the flowering time were identified using mutants through genetic analyses in many previous studies (Yano et al. 2001). For the last decade, thanks to the application of marker approaches, it allowed for the detection of several critical genes involved in flowering time in rice (Yano and Sasaki 1997) and the estimation of gene functions affecting early- or late-flowering phenotypes became possible as well as the analysis of epistatic interactions among quantitative trait loci (QTL; Xiao et al. 1996).

To date, 15 QTL were shown to control heading date in rice and four QTL among these were isolated by a map-based cloning approach (Uga et al. 2007). *Hd1*, known as a transcription factor, was identified (Yano et al. 2001) and has similar expression pattern like *CO* in *Arabidopsis* (Izawa et al. 2003). *Hd3a*, an ortholog of *FT* in *Arabidopsis*, was found on chromosome 6. Under short-day (SD) conditions, this gene promoted flowering (Kojima et al. 2002; Yano and Sasaki 1997). *Hd6*, known as encoding a casein kinase II subunit, was also isolated (Yamamoto et al. 2000). *Se5*, a homologous gene of *Arabidopsis HY1*, was found to encode for a heme-oxygenase and cause late flowering under long-day (LD) condition (Izawa et al. 2000). Except for these four QTL, the others remain unclear yet.

QTL identification is a typical time-consuming, labor- and cost-intensive work. In most cases, RIL (recombinant inbred line), NIL (near isogenic line), and/or DHL (double haploid line) populations are preferred over F_2 populations because RIL and DHL make it possible to replicate the QTL analysis under different environmental conditions (Yoo et al. 2007). But F_2 populations are still being used for QTL identification in spite of these shortcomings, because it is cost-saving compared to other lines. Indeed, five QTL among a total of 15 QTL for flowering time in rice were detected with this F_2 population (Lin et al. 2002; Lin et al. 1998; Yamamoto et al. 2000). Another useful approach to improve the efficiency of

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QTL mapping is to apply selective genotyping method which uses only individuals exhibiting high and low phenotypic extremes (Darvasi and Soller 1992). This selective genotyping method has been used in various species to reduce cost, time, and labor. Nevertheless, to detect QTL still remains hard work due to methodological difficulties and complex epistasis among several genes (Yamamoto et al. 2000).

One of the important factors affecting growth and heading date in rice is the response to seasonal changes in photoperiod. Rice is basically a SD plant whose flowering is accelerated under SD conditions. However, rice plants can flower under LD conditions due to its facultative character, even though days-to-heading of rice is much delayed. The sensitivity of response to photoperiod is increasing with the order of early-maturing, middle-maturing, and late-maturing plants (Lee et al. 2006). Seolakbyeo, one of the early-maturing cultivars, was almost insensitive to photoperiod compared to other regular cultivars (Choi et al. 1983). The photoperiod insensitivity to day lengths may help flowering in the region under constant LD conditions. Here, we report two useful QTL for early heading date (EHD) through QTL analysis with H143 line showing extreme EHD in rice. These QTL were closely related to the previously reported QTL and the combination of certain alleles of these two genes contributed to early heading in H143 background. In addition, the test of photoperiod response revealed that H143 is more insensitive than the early maturing rice cultivar 'Seolakbyeo', indicating that H143 can be useful as a breeding source for the adaptation of rice to improper photoperiodic conditions.

Materials and Methods

Plant material and field experiments

H143 showing EHD was derived from the cross between the spontaneous mutant N11 and H75. To analyze QTL conferring EHD, H143 was crossed with 'Milyang23 (M23)' showing relatively late flowering in order to generate F_2 population. Heading date was investigated with 1365 F_2 plants obtained from the cross. Among the F_2 individuals derived from the cross of H143/M23, 44 F_2 individuals showing extreme phenotypes (22 extremely early-flowering and 22 quite late-flowering phenotypes) were used to identify major QTL. For the heading date analysis in the same *japonica* subspecies, H143 was also crossed with 'Dongjinbyeo'. A total of 1,009 F_2 plants were measured for heading date. The F_2 populations, F_1s, and parental lines from two crosses were planted in the rice-growing season in 2002 in the experimental farm of Seoul National University, Suwon, Korea. Heading date was recorded at the day when the spikelets of a first panicle were shown.

DNA markers and pooling test

We extracted genomic DNA from leaves of F_2 and parental lines using the CTAB method (Murray and Thompson 1980). Fifty-eight SSR markers for pooling test covering all 12 rice chromosomes were used and 44 F_2 individuals exhibiting extreme heading date were used to obtain pooled DNA samples. Forty F_2 individuals were separated into two groups according to their heading date, designated as Pool I and Pool II, respectively.

Test of day length response

To investigate the change of heading date, Seolakbyeo, one of the early-flowering regular rice cultivars, and H143 line were grown in the growth chamber. Growth conditions were set with 16-h light/8-h dark (16L/8D) for LD and 8L/16D for SD conditions, and temperature was fixed at constant 30°C. Seedlings were planted into a Wagner pot of 1/5,000 and grown under the 5-4-4 kg/10a (N-P-K) fertiliza-

Table 1. QTLs for early heading date, resolved using Q-gene 3.06 in the F_2 population derived from the H143/M23 cross with the LOD thresholds computed through permutation tests $P = 0.05$ and 0.01.

QTL	Chr[a]	Interval[b]	LOD	A[c]	D[c]	D/A	R^2 (%)[d]	permutation[e]	
								95%	99%
EH7-1	7	RM7110-RM346	5.7	-16.14	19.69	-1.22	44.9	2.51	3.26
EH7-2	7	RM420-RM3555	6.81	-23.21	-7.48	0.32	50.9		
	Total						57.8		

[a,b] Chromosome number and marker intervals.
[c] *A* and *D* are additive and dominant effects, and the negative values indicate the alleles from M143 that decrease the trait score.
[d] Phenotypic variation rate explained by the detected QTLs for the trait.
[e] The numbers in each of the cells indicate the LOD thresholds that are computed through permutation tests $P = 0.05$ and 0.01.

Table 2. Summary of PCR-based rice SSR markers on chromosome 7 used in the QTL mapping.

Marker name	Forward primer	Reverse primer
RM427	TTGAGCTGATGAGAGTTGGTTGC	CTGTCACTAGCTCTGCCCTGACC
RM481	TAGCTAGCCGATTGAATGGC	CTCCACCTCCTATGTTGTTG
RM6574	AACCTCGAATTCCTTGGGAG	TTCGACTCCAAGGAGTGCTC
RM7110	GGCGATCTCTGTGTTTATTG	ATTAACCGGTTGAGATGGTG
RM346	CGAGAGAGCCCATAACTACG	ACAAGACGACGAGGAGGGAC
RM455	AACAACCCACCACCTGTCTC	AGAAGGAAAAGGGCTCGATC
RM234	ACAGTATCCAAGGCCCTGG	CACGTGAGACAAAGACGGAG
RM5455	CTCGGCCTGACTAGTCGATC	TGATGGCGATCCTGTGATAG
RM3555	TGGAAGTTTCCTGGCGATAG	TGGTTGGACTGAAAAGTCCC
RM420	CCTCTCACTCTGCCTGCTCTACC	TCTCTAACTCTTGAGTGACAGCAACC

tion. Heading date was recorded at the day when the spikelets of a first panicle first became visible.

Linkage mapping of QTL analysis

A linkage map was constructed using Mapmaker 3.0 (Lander et al. 1987; Lincoln et al. 1993) and MapChart 2.0. Distances between markers are given in centiMorgans (cM) using the Kosambi map function (Kosambi 1994) and the order of markers was established by three-point analysis. Qgene 3.06 software was used to detect QTL for EHD and LOD score 3.0 was set to examine putative QTL in the analysis of QTL map (Nelson 1997). The proportion of phenotypic variation explained by each QTL was calculated as the R^2 value, and the degree of dominance of each QTL was estimated as the ratio of dominance effect to additive effect (D/A).

Results and Discussion

Characterization of H143 and phenotypic variation

The days-to-heading of H143 was much earlier than other regular early-flowering cultivars such as Seolakbyeo. First, we investigated heading date of H143 by comparing with Seolakbyeo. Under natural growing conditions, H143 flowered 10 days earlier than Seolakbyeo (Fig. 1). In addition, H143 had fewer tillers than that of Seolakbyeo. These results suggest that H143 went from vegetative to reproductive stage without sufficient vegetative growth. Meanwhile, a large difference in the days of heading between H143 and Milyang23 (M23) was observed (Fig. 1) and the segregation pattern was investigated by crossing these two varieties (Fig. 2A). Means of days to heading of H143 and M23 were 71 and 115 days, respectively, and the gap of heading date in two parents was 44 days. The average of F_1 plant heading dates was 104 days which is closer to M23 than H143. The bias of the heading date to that of M23 in F_1 plants suggests that the EHD trait of H143 may be recessive and controlled by a few major QTL. The heading dates in F_2 lines ranged from 63 to 133 days and showed transgressive segregation, indicating that multiple loci are involved. We also checked the segregation mode of heading date in the cross of same subspecies, *japonica/japonica*. In the F_2 population of the H143/Dongjinbyeo cross, the variation of heading date was reduced from 83 and 130 days compared to H143/M23 (Fig. 2B). This result suggests that in the cross between the same subspecies, a small variation was observed for days to heading in the F_2 population because genetic variation between parental plants for heading date is low.

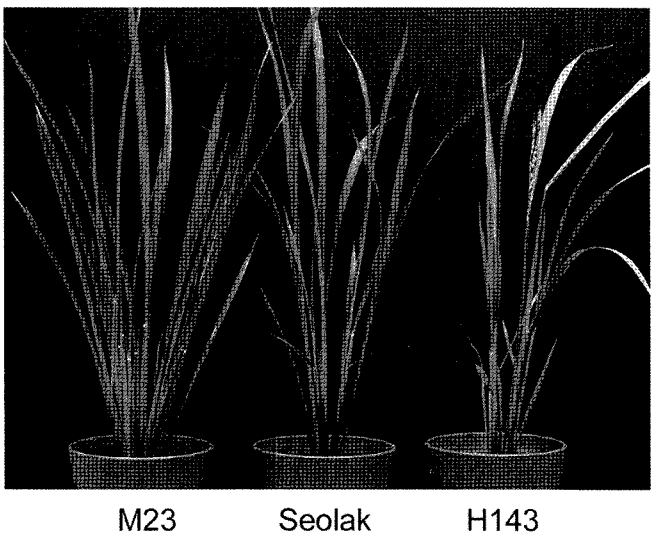

Fig. 1. Comparison of days-to-heading of three cultivars. Extreme early maturing plant, H143, flowered 10 days earlier than Seolakbyeo (Seolak), an early maturing cultivar, and 44 days earlier than Milyang23 (M23), a middle maturing cultivar. All plants were grown during the growing season in the field conditions and moved to pots for photographs.

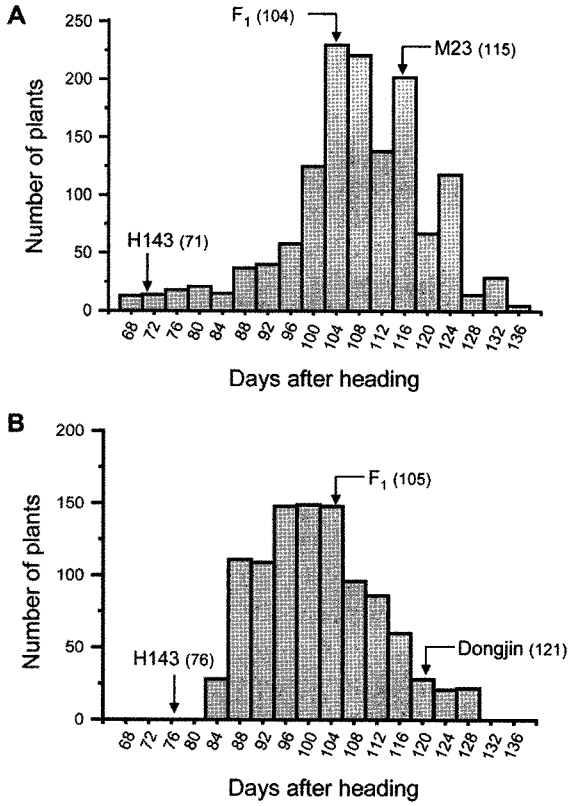

Fig. 2. Frequency distribution of days-to-heading in two F_2 populations. Days of heading of parents, F_1 and F_2 plants were measured when panicles were emerged. (A) F_2 population derived from the H143/M23 (M23) cross. (B) F_2 population derived from the H143/Dongjinbyeo (Dongjin) cross. The numbers in parenthesis represent average days-to-heading of each plant.

Pooled-sample approach

One of the strategies to construction of genetic maps was a pooled-sample approach (Churchill et al. 1993). First, we conducted a pooling test to probe a linked chromosomal location for early flowering time by using 58 SSR markers covering all 12 chromosomes. In this experiment, we detected a highly linked marker, RM 7110 on chromosome 7, while other regions were found to be only slightly linked or not. The SSR markers, RM38 on chromosome 3 and RM421 on chromosome 5, show that these regions are not linked to EHD (Fig. 3). Through further tests using more SSR markers on chromosome 7, one additional SSR marker, RM420, was found to be related to EHD character, indicating that QTL analysis needs to focus on chromosome 7.

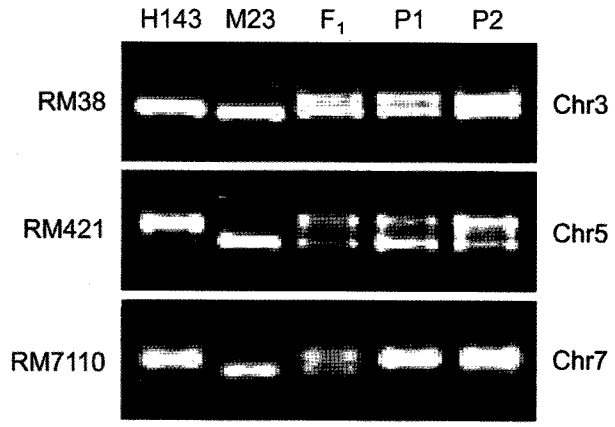

Fig. 3. Pooling test for detecting the linked chromosomal region using 55 SSR markers over 12 chromosomes with two groups of pooled genomic DNAs. Unlinked two SSR markers, RM38 and RM421, were represented as an experimental control. RM7110 represents highly biased band patterns into H143 allele. P1, pooled DNA of 1 to 20th plants according to heading date; P2, pooled DNA of 21 to 40th plants according to heading date.

Linkage map and QTL analysis

Among 15 SSR markers, 10 SSR markers with a good coverage on chromosome 7 were used to construct a linkage map using Mapmaker 3.0 (Fig. 4A). The map covered 111.6 cM with an average distance of 12.4 cM between markers, which is less than the minimum required level, 20 cM, for QTL mapping (Lander and Botstein 1989). Comparison between the resulting linkage maps and the previous maps (Temnykh et al. 2001) revealed that all of the markers were located in the expected order on rice chromosome 7. For the QTL analysis, a total of 44 F_2 individuals showing extreme phenotype, 22 of early heading date and 22 of late heading date, were selected from F_2 populations and genotyped with 10 SSR markers (Table 2). Interval mapping identified a total of two major QTL above the LOD thresholds that were com-

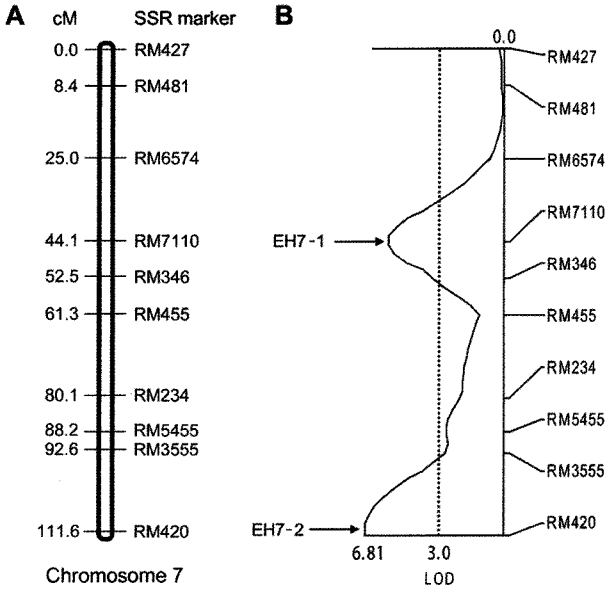

Fig. 4. Linkage map and QTL positions detected on chromosome 7 in the F_2 population derived from the H143/M23 cross. (A) Linkage map constructed with 10 SSR markers on chromosome 7 and 44 F_2 individuals showing early and late heading date. (B) QTL positions developed using Q-gene 3.06 software. The names of the QTLs are indicated to the left of the chromosome. A LOD dotted line of 3.0 is shown in the QTL curve. EH, early heading.

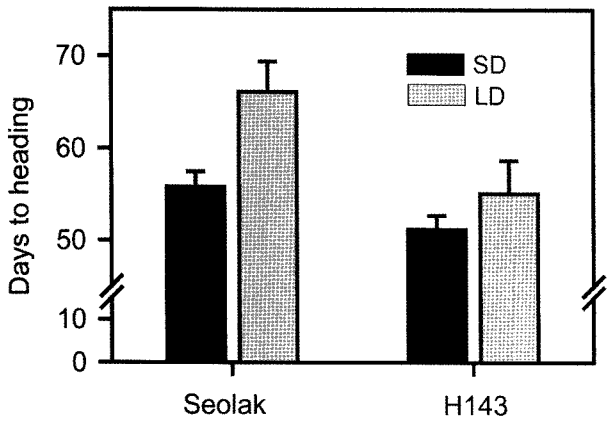

Fig. 5. Changes of days to heading of Seolakbyeo and H143 according to photoperiod transition. Seolakbyeo and H143 were grown under SD and LD conditions in the growth chambers. H143 flowered 3.9 days earlier in SD than in LD conditions, while Seolakbyeo flowered 10.3 days earlier in SD than in LD conditions.

puted through permutation test at $P = 0.05$ and 0.01 (Table 1 and Fig. 4) for EHD. One QTL, EH7-1, was detected to be linked to SSR marker, RM7110, with 44.9% of the high phenotypic variation and the other QTL, EH7-2 was closely linked to RM420 with 50.9% of phenotypic variation (Table 1 and Fig. 4B). The total phenotypic variation accounted for by these two QTL reached 58%. At these two QTL, the alleles from H143 genotype had a negative effect on heading date. Additive effects of H143 allele for EH7-1 and EH7-2 were approximately 16.1 and 23.2 days, respectively. The results indicated that EHD in H143 background was mainly caused by these two QTL, EH7-1 and EH7-2, and EH7-1 contributed more to the EHD phenotype. Interestingly, these loci are consistent with the QTL which were previously reported for EHD, showing that both qDTH-7-1 and qDTH-7-2 were detected on chromosome 7 from the QTL analysis in the BC_1F_1 population and observed epistatic interaction between them (Fujino and Sekiguchi 2005). The recessive alleles of these two genes contributed to EHD. It seems that EH7-1 and EH7-2 are the same QTL as qDTH-7-1 and qDTH-7-1, because they are closely linked and highly linked to *Hd4* and *Hd2*, respectively. *Hd4* was reported to be linked to RM7110 on chromosome 7 (Fujino and Sekiguchi 2005) and *Hd2* was found to be near the markers C213 and RM1306 on chromosome 7 (Lin et al. 2003; Yano and Sasaki 1997). In our study, *Hd4* was closely linked to RM7110 and *Hd2* was highly linked to RM420 existing between C213 and RM1306, indicating that these QTL may be the same loci. These loci have been consistently identified from different crosses of various genetic backgrounds, suggesting that these loci may be used for the rice breeding programs of early-heading plants.

Test of day length response

When compared to Seolakbyeo, one of the typical early-heading cultivars, the days-to-heading of H143 was 10 days earlier than that of Seolakbyeo in the field conditions. Rice did not flower only in the condition of SD, but also flowered in LD condition, because rice is basically a facultative plant (Halevy et al. 2001). To understand whether day length influences flowering in H143 background, Seolakbyeo and H143 were grown at both SD and LD conditions in the environmentally well-controlled chambers. In SD conditions, H143 came into flower at 51.2 days after germination and Seolakbyeo at 55.8 days, while in LD condition, the day-to-heading took 55.1 days for H143 and 66.1 days for Seolakbyeo (Fig. 5). Days-to-heading of H143 was reduced 3.9 days in LD compared to SD conditions, while that of Seolakbyeo was reduced 10.3 days, indicating that H143 is less sensitive than Seolakbyeo in response to photoperiod. The results suggest that H143 can be used as a breeding source for the development of rice varieties suitable for cultivation in regions characterized by inappropriate day length.

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