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Tagging quantitative trait loci for heading date and plant height in important breeding parents of rice (*Oryza sativa*)

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Abstract Heading date and plant height are important determinants for plant growth rate. In this study, simple sequence repeat markers were used to tag quantitative trait loci (QTL) using a recombinant inbred line mapping population derived from two important breeding parents, genetic stock Kaybonnet*lpa*1-1 and *indica* cultivar Zhe733, using data collected under field and greenhouse conditions. Interval mapping, composite interval mapping, and multiple interval mapping were performed to map QTL for heading date and plant height, and to identify epistatic interactions between the QTL. *qHD3.1* on chromosome 3 from KBNT*lpa*1-1 had the largest effect on heading date contributing an average of 28.4 % of the total phenotypic variation. *qHD7.1*, *7.2*, and *8.1* also had a significant contribution to heading date from Zhe733 averaging 8.1, 12.8, and 12.8 % of the phenotypic variance, respectively, and there was a positive additive-by-additive epistatic interaction between *qHD7.1* and *qHD8.1*. QTL, *qPHT1.1* and *qPHT3.1*, for plant height were detected on chromosomes 1 and 3, respectively. *qPHT1.1* contributed the largest effect representing 38.2 % of the total phenotypic variation. Comparison of the QTL identified in our study with previous results revealed that the chromosomal locations for QTL coincided closely with positions reported previously in other rice populations worldwide, suggesting that these QTL have coevolved and become domesticated. The tightly linked SSR markers that flank these QTL should be desirable for tagging heading date and plant height genes and facilitating their incorporation into advanced breeding lines using marker assisted selection.

Keywords Heading date · Marker-assisted selection · Plant height · Quantitative trait loci · Rice (*Oryza sativa*)

Abbreviations

CIM	Composite interval mapping
IM	Interval mapping
KBNT*lpa*1-1	Kaybonnet*lpa*1-1
LOD	Logarithm of odds

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MAS	Marker assisted selection
MIM	Multiple interval mapping
QTL	Quantitative trait loci
RIL	Recombinant inbred line
SSR	Simple sequence repeat

Introduction

Heading date and plant height are two of the most important traits associated with yield potential in rice and they are controlled by quantitative trait loci (QTL). Appropriate heading date and plant height are therefore prerequisites for developing high-yielding varieties. Operationally, traditional selection based on phenotype for heading date and plant height relies on field plot experiments during crop growing seasons. In breeding programs, this is often problematic when natural and human resources are limited. With the advent of molecular marker techniques as well as the availability of saturated molecular maps and rice genome sequence information, it is now possible to identify and localize genes controlling complex traits for heading date and plant height.

For heading date, studies have been reported in various rice populations (Li et al. 1995; Yano et al. 1997; Takahashi et al. 2001; Lin et al. 2002; Monna et al. 2002; Lin et al. 2011). Two QTL for heading date, *Qhd3a* on chromosome 3 and *Qhd8a* on chromosome 8, were found in the population of Lemont/Teqing (Li et al. 1995) and *Ghd8* was recently cloned (Yan et al. 2011). Fifteen QTL conditioning heading date, *Hd1–Hd3a* and *Hd3b–Hd14*, have been reported from a cross between a *japonica* variety Nipponbare and an *indica* variety Kasalath (Yano et al. 1997; Lin et al. 1998; Takahashi et al. 2001; Lin et al. 2002; Monna et al. 2002) and mapped on chromosomes 2, 3, 4, 6, 7, 8, 10, and 12. Of these *Hd1*, *Hd3a*, and *Hd6* have been successfully cloned in rice (Yano et al. 2000; Takahashi et al. 2001; Kojima et al. 2002). In addition, two other heading date QTL, *Ehd1* and *Ghd7*, have been cloned (Doi et al. 2004; Xue et al. 2008). It is also well known that several of these QTL interact epistatically including *Hd2* with *Ghd7* and *Hd5* with *Hd1* (Lin et al. 2003; Shibaya et al. 2011). In addition, it has been shown that *Ghd7* affects expression of *Ehd1* and *Hd3a* (Xue et al. 2008).

A number of studies have been performed to determine QTL for plant height in rice. The semi-dwarf gene *sd-1* was first characterized, and subsequently cloned (Hedden 2003). In addition, several studies have reported QTL controlling plant height in rice (Li et al. 1995; Huang et al. 1996; Ashikari et al. 1999; Yu et al. 2002; He et al. 2005). Huang et al. (1996) identified 13 QTL for plant height using five rice populations. Ashikari et al. (1999) cloned a gene for the gibberellins-insensitive dwarf mutation in *Dwarf 1* (*D1*) encoding the α-subunit of the GTP-binding protein. More recently, ten QTL for plant height on nine of the 12 chromosomes have been identified by using single segment substitution lines (He et al. 2005). Heading date gene *Ghd7* has also been reported to increase plant height when overexpressed under long-day conditions (Xue et al. 2008).

The *indica* cultivar Zhe733 (PI 629016) and genetic stock Kaybonnet*lpa*1-1 (KBNT*lpa*1-1, PI 632282) have been important resources for rice breeding for improved yield, nutrient quality, and disease resistance in the USA and worldwide (Rutger and Tai 2005). Zhe733 and KBNT*lpa*1-1 are known to possess distinct phenotypic differences for plant height and heading dates; therefore, they are ideal for introducing different QTL. It is important to note that Zhe733 is a high yielding and early maturing semi-dwarf cultivar that can be grown in the southern USA, and KBNT*lpa*1-1 is a mutant of Kaybonnet that has lower phytic acid content than its parent resulting in increased mineral nutritional value. A mapping population (K/Z) of the cross of KBNT*lpa*1-1 and Zhe733with a linkage map was previously constructed and used for mapping QTL for resistance to rice sheath blight and blast disease (Liu et al. 2008; Lee et al. 2009; Jia and Liu 2011). However, QTL for heading date and plant height present in K/Z population and their potential importance in US rice breeding have not been determined. Thus, the objective of this study was to identify QTL for heading date and plant height in KBNT*lpa*1-1 and Zhe733 using the K/Z mapping population grown in the rice fields and in a greenhouse.

Materials and methods

Plant materials, experimental design, and phenotyping

The *indica* semi-dwarf cultivar Zhe733 and the genetic stock KBNT*lpa*1-1 were used as the parents

in this study. KBNT*lpa*1-1 is the first low-phytate mutant induced in the rice cultivar Kaybonnet by γ-irradiation (Bryant et al. 2005). A total of 255 F_{10-11} recombinant inbred lines (RILs) were used for determining the chromosomal locations of QTL for heading date and plant height. The F_{10-11} RILs together with two parents were transplanted into the field at the University of Arkansas Rice Research and Extension Center, Stuttgart, AR, USA in the 2002, 2003, and 2004 rice-growing seasons. Each RIL was randomly assigned to a plot of 0.46 m^2. Data were collected from the field plots for days to heading (days from planting to 50 % heading) and plant height (distance, cm, from soil surface to panicle tip before harvesting). The average of the data for heading date and plant height from each year was used for QTL mapping. In order to detect QTL under highly controlled environmental conditions the same population was grown in a greenhouse in 2011. The greenhouse experiment was designed as random complete block with three replications, one set of replicates per bench. Each pot was filled with sterilized local clay soil and 3–5 seeds per line were sown. One plant per pot was maintained until heading. Data collected from sowing date, heading date, and plant height for each pot and each line; the average from the three replications was used for QTL analysis.

Simple sequence repeat (SSR) analysis

One hundred and eighteen SSR markers in 255 F_{10-11} RILs were analyzed for mapping QTL for heading date and plant height in the K/Z population. PCRs were performed as previously described by Liu et al. (2008), except the reaction volume was changed to 25 μL. Amplified products were diluted between 40 and 2000×, and 2 μl of the diluted product was added to 9 μL of formamide containing ROX-labeled size standard. PCR products from three primer pairs with different size ranges and fluorophore labels were combined to determine the sizes of SSR alleles. The reaction was run on an ABI Prism 3700 DNA Analyzer according to the manufacturer's instructions (Applied Biosystems, Foster City, CA, USA). Fragment sizing and SSR marker genotype analysis were performed using GeneScan® and Geneotyper® software Version 3.7 (Applied Biosystems, Foster City, CA, USA).

Construction of linkage map and QTL analysis

A linkage map of 118 polymorphic SSR markers was constructed using JoinMap® 4 (Van Ooijen 2006). A genetic map with cumulative cM distances was generated using Kosambi's mapping function from 255 RILs of 'Zhe733' × 'KBNT*lpa*1-1'. QTL analysis was performed using the phenotypic and genotypic data of heading date and plant height from 255 F_{10-11} RILs. Interval mapping, composite interval mapping (CIM), and multiple interval mapping (MIM) were performed to identify SSR markers associated with heading date and plant height using the software Windows QTL Cartographer 2.5 (Wang et al. 2007). The value of additive genetic effect and phenotypic variation explained by each putative QTL were obtained from the program concurrently. QTL positions on chromosomes were determined using a logarithm of odds (LOD) threshold of 3.2–3.5 for plant height and heading date for field data and 2.9 for heading date and 3.0 for plant height for greenhouse data, as determined by performing 1,000 permutations, for an experiment-wise significance level of 0.05 using CIM. QTL were named following the gene nomenclature system for rice (McCouch 2008).

Results

Phenotypic variation for heading date and plant height

The overall days to heading for the F_{10-11} RIL population from KBNT*lpa*1-1 and Zhe733 was 66–110 days (Fig. 1). The difference of heading date between parents was approximately 20 days with KBNT*lpa*1-1 having a heading date of 95 ± 5.7 days and Zhe733 having a heading date of 75 ± 5.7 days. The plant height of KBNT*lpa*1-1 (104 cm) and Zhe733 (90 cm) differed by approximately 14 cm while the height of F_{10-11} RIL individuals varied from 78 to 170 cm (Fig. 1). A number of RILs with increased plant height phenotype was observed in the F_{10-11} population. In the RIL population, heading date and plant height showed a two-peak distribution with a large transgressive segregation over the two parents (Fig. 1), indicating quantitative inheritance.

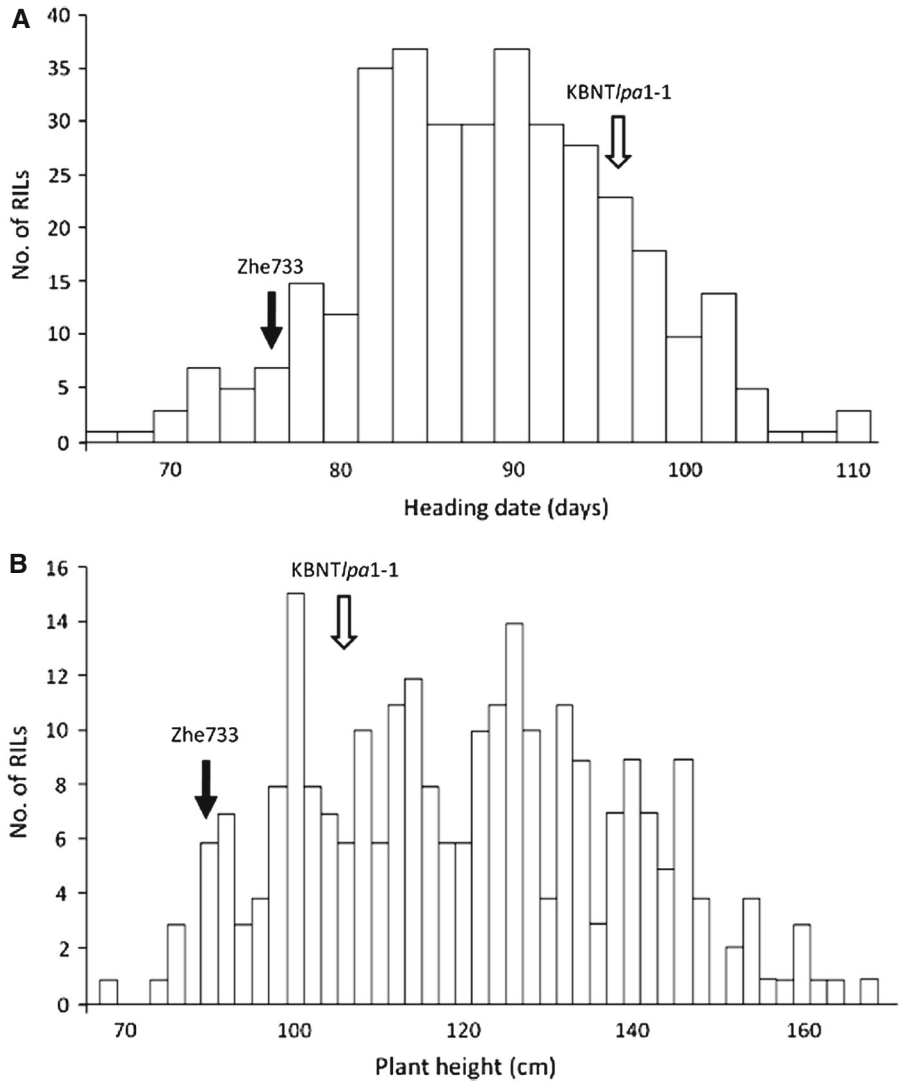

Fig. 1 Adjusted means frequency distributions for heading date (**a**) and plant height (**b**) of 255 F_{10-11} RIL population from the cross "KBNT*lpa1-1* and Zhe733". Plant height was assessed for 2 years (2002 and 2003) and heading date was assessed for 3 years (2002, 2003, and 2004) in the field plot located at Stuttgart, Arkansas. *Arrows* indicate the adjusted mean values of the parents, Zhe733 and KBNT*lpa1-1*

QTL for heading date

Using 118 SSR markers in the K/Z population, CIM and MIM detected six QTL, *qHD3.1, qHD7.1*, *qHD7.2*, *qHD8.1*, *qHD8.2,* and *qHD10.1*, for heading date (LOD of 2.9–32.8) from field data collected in 2002, 2003, and 2004 and greenhouse data collected in 2011 (Fig. 2). Only QTL identified in three years data for heading date and two years data for plant height were considered valid. The first QTL, *qHD3.1*'s peak location was 3.8 cM on chromosome 3 between RM132 and RM231 and provided the largest phenotypic effect and contributed an average of 28.4 % of total variation for heading date. Nine known QTL were located near *qHD3.1* (Fig. 2). Two other QTL, *qHD7.2* and *qHD8.1*, on chromosomes 7 and 8, respectively, provided the second largest effects both contributing an average of 12.8 % of the phenotypic variation. *qHD8.2* on chromosome 8 and *qHD10.1* on chromosome 10 contributed the least phenotypic variation with 5.4 and 7.4 %, respectively (Table 1). The estimated additive genetic effects resulted in an average decrease of heading date by 1.7–4.2 days (Table 1). The earliness allele at *qHD3.1* was contributed by KBNT*lpa1-1* and alleles at *qHD7.1, qHD7.2, qHD8.1,* and *qHD8.2* were contributed by Zhe733 for the earliness of heading date. *qHD10.1* had different effects in the field and greenhouse with the KBNT*lpa1-1* allele conferring earliness

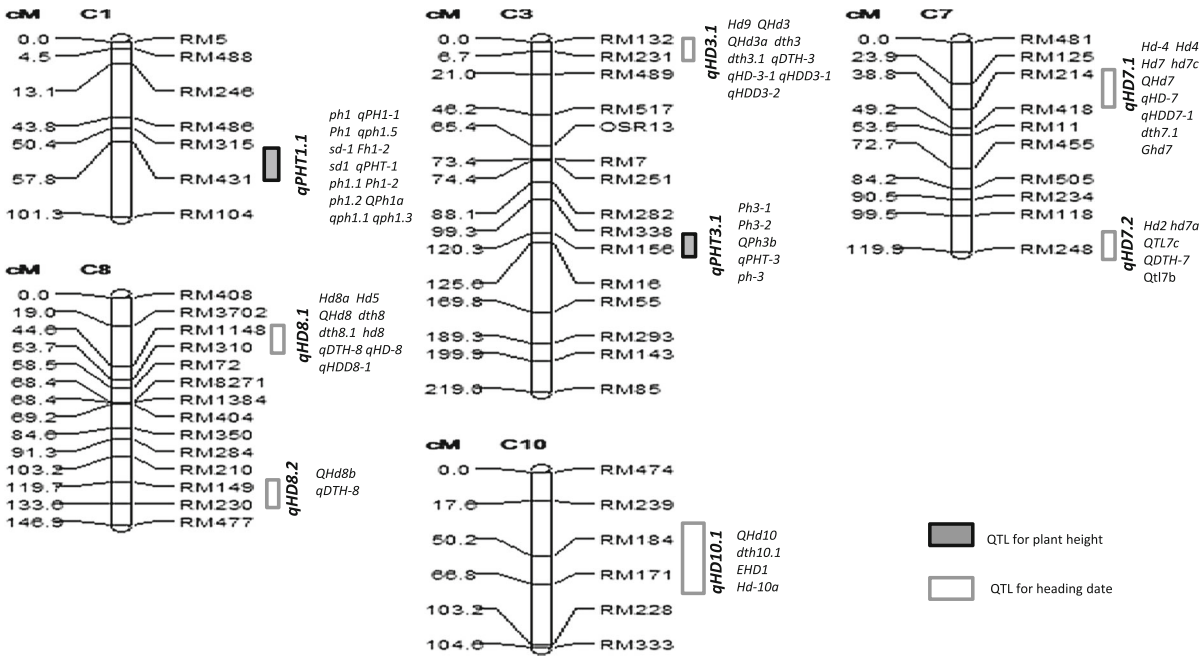

Fig. 2 Molecular genetic maps of QTL controlling plant height and heading date in 255 $F_{10–11}$ RIL population from the cross "KBNT*lpa1-1* and Zhe733". Six QTL for heading dates and two QTL for plant height were detected in this study. Previously identified QTL from different mapping populations are listed next to each QTL. The significance threshold of the logarithm of odds (LOD) was between 2.9 and 3.5 for the detection of putative QTL based on the threshold established by 1,000 permutations

in the greenhouse and the Zhe733 allele conferring earliness in the field. The location of *qHD10.1* was slightly different between the greenhouse and field data, possibly indicating that these are two different *QTL*. A positive additive-by-additive epistatic interaction was identified between *qHD7.1* and *qHD8.1*, indicating that the Zhe733 alleles at both loci together will reduce heading date by an additional 2 days bringing a total of 6 day reduction.

The chromosomal positions of *qHD3.1* and *qHD7.1* were highly similar between field data and greenhouse data. Three QTL, *qHD7.2*, *qHD8.1*, and *qHD8.2*, were only detected in the field experiment. *qHD10.1's* position was slightly different between the field and greenhouse. Similar additive effect was observed for *qHD3.1* and *qHD7.1*; however, opposite additive effect was observed for *qHD10.1* (Tables 1 and 2).

QTL for plant height

Two QTL, *qPHT1.1* and *qPHT3.1*, for plant height were identified on chromosomes 1 and 3 (Fig. 2), respectively. *qPHT1.1* on chromosome 1 between flanking SSR markers, RM315 and RM431, had the largest effect contributing about 38.2 % of total phenotypic variation for plant height (LOD = 34.9) with the Zhe733 allele, conferring a reduction of 11.8 cm in height. Fourteen known QTL, including semi-dwarf gene *sd-1*, were located near *qPHT1.1* (Fig. 2). *qPHT3.1* explained 7.2 % of phenotypic variation (LOD = 5.9) and caused a reduction of 7.8 cm in plant height. Five known QTL were located near *qPHT3.1* (Fig. 2). The height-reducing allele from *qPHT1.1* was contributed by parent Zhe733 whereas *qPHT3.1* was contributed by KBNT*lpa1-1* (Table 1). No epistasis was observed between these two QTL.

All plant height QTL identified in the field were also identified in our greenhouse experiment (Table 2). The chromosomal positions of *qPHT1.1* and *qPHT3.1* were highly similar between each year of field and greenhouse data. Similar additive effects were observed for *qPHT1.1* and *qPHT3.1*.

Discussion

Both heading date and plant height are major determinants of the growth rate of rice and are known to be

Table 1 QTL for heading date and plant height detected by multiple interval mapping analysis in the F_{10-11} RIL population of KBNT*lpa*1-1/Zhe733

QTL	Chr.	SSR marker[b]	Position (cM)[c]	LOD[d]	PVE[e]	Add (%)[f]	Epistasis
Heading date[a]							
qHD3.1	3	RM132–RM231	3.8 ± 1.7[g]	21.7 ± 4.6[g]	28.4 ± 9.6[g]	4.2 ± 1.0[g] (20)	
qHD7.1	7	RM214	38.5 ± 0..5	11.2 ± 7.0	8.1 ± 2.9	−2.8 ± 1.0 (8)	AA[j]
qHD7.2	7	RM248	116.8 ± 2.5	18.8 ± 8.5	12.8 ± 7.4	−2.4 ± 0.1 (10)	
qHD8.1	8	RM1148–RM310	46.6 ± 0	12.4 ± 3.9	12.8 ± 1.9	−3.8 ± 0.5 (8)	AA[j]
qHD8.2	8	RM149–RM230	126.7 ± 1.7	6.2 ± 1.5	5.4 ± 2.6	−1.7 ± 0.2 (6)	
qHD10.1	10	RM184–RM171	61.5 ± 8.2	6.8 ± 1.5	15.0[h]	2.8[i]	
Plant height, cm[a]							
qPHT1.1	1	RM315	54.4 ± 1.0[g]	34.9 ± 8.6[g]	38.2 ± 1.9[g]	−11.7 ± 1.6[g] (42)	
qPHT3.1	3	RM338	109.3 ± 2.8	5.9 ± 1.2	7.8 ± 1.6	7.2 ± 2.9 (8)	

[a] Estimates of QTL with significant effects on days to heading and plant height were evaluated in two (plant height) or three (heading date) year field experiments and in the greenhouse

[b] The interval or closest markers associated with each QTL

[c] Position indicates the mean chromosome-peak position (cM) of the highest LOD score for the respective QTL

[d] LOD is the log-likelihood ratio at the QTL position and maximum LOD score for a given marker interval

[e] PVE is the percent phenotypic variation explained by an individual QTL. We used R^2 values from MIM to represent this value.

[f] Standard deviations (SD) were computed from greenhouse and different years field experiments; 2002, 2004, and 2011 (greenhouse) for heading date and 2002, 2003, and 2011 (greenhouse) for height

[g] The value of additive genetic effect associated with the Zhe733 allele. A negative value indicates that the Zhe733 allele decreases the value of the trait and a positive value means that the Zhe733 allele increases the value of the trait

[h] Effect was opposite and location did not overlap for *qHD10.1* Since PVE was higher for greenhouse data, we are showing PVE for greenhouse only; the PVE for field was an average of 3.6

[i] Data for greenhouse only; field effect was an average of −1.4

[j] An additive-by-additive epistatic interaction, $p < 0.001$, was observed between *qHD7.1* and *qHD8.1* in which the presence of the Zhe allele at both loci resulted in an additional 2 ± 0.28 days reduction in heading date

Table 2 Presence and location of QTL for plant height and heading date from the field and greenhouse data from different years and conditions

QTL	Field 2002 (cM)	Field 2003 (cM)	Field 2004 (cM)	Greenhouse 2011 (cM)
qPHT1.1	51.1–56.2	52.8–56.6	[a]	52.3–56.6
qPHT3.1	Not found	100.7–113.8	[a]	102.5–115.1
qHD3.1	1.1–5.4	0–4.3	0–5.8	0–7.0
qHD7.1	33.2–43.1	37.5–43.8	32.7–43.3	29.5–48.7
qHD7.2	109.9–118.9	112.1–119.6	116.5–119.4	Not found
qHD8.1	41.5–52.7	36.7–50.8	32.3–52.0	Not found
qHD8.2	113.8–132.3	120.7–130.1	119.4–133.4	Not found
qHD10.1	Not found	57.4–76.2	59.2–82.3	36.2–56.9

[a] No height data taken in 2004

influenced by the environment. The present study identified six heading date QTL and two plant height QTL in KBNT*lpa*1-1 and Zhe733 using phenotypical data from controlled (greenhouse) and uncontrolled (multiyear field) conditions. Most of the QTL identified in the field study were also identified in a replicated greenhouse study. These QTL correlate with previously reported heading date and plant height

Table 3 Comparisons of QTL affecting heading date and plant height identified in the present study to QTL reported in previous studies

QTL	Chr.	Flanking markers	Physical position (Mb)[a]	No. of QTL[b]	Reference[e]
Heading date					
qHD3.1	3	RM132–RM231	1–2.5 Mb	9	Hittalmani et al. (2003), Mei et al. (2003), Yano et al. (2001), Thomson et al. (2003)
qHD7.1	7	RM125–RM418[c]	5.4–18.1 Mb	9	Yano et al. (1997), Thomson et al. (2003), You et al. (2006)
qHD7.2	7	RM118–RM248	26.6–29.3 Mb	5	Lin et al. (2002)
qHD8.1	8	RM3702–RM310	1.1–5.1 Mb	9	Lin et al. (2003), Yano et al. (1997)
qHD8.2	8	RM210–RM230	22.3–25.8 Mb	2	Li et al. (2003), Suh et al. 2005
qHD10.1	10	RM239–RM171[d]	9.3–18.8 Mb	4	Doi et al. (2004), Li et al. (2003), Thomson et al. (2003), Lu et al. (1997)
Plant height					
qPHT1.1	1	RM315–RM431	36.7–38.9 Mb	14	Yan et al. (1998), He et al. (2001), Hittalmani et al. (2003), Septiningsih et al. (2003), Thomson et al. (2003), Lin et al. (2011)
qPHT3.1	3	RM338–RM156	13.2–17.7 Mb	5	Hemamalini et al. (2000)

[a] The physical position of the QTL are based on *O. sativa* ssp. *japonica* cv. Nipponbare sequence. Unannotated SSRs were placed on the Nipponbare map by blasting the primers; verifying the presence of the appropriate repeat in that genomic region

[b] Information of the corresponding QTL symbols and chromosome locations were obtained from Gramene QTL database (http://www.gramene.org/qtl/) unless the data was not present in Gramene. Data from references were used in those cases. QTL details are described in Fig. 2

[c] Location of *qHD7.1* in the greenhouse was slightly different from the field, flanking markers were RM125–RM11 placing the QTL from 5.4–19.3 Mb

[d] Location of *qHD10.1* in the field was different from greenhouse. Flanking markers were RM184–RM228

[e] References were cited if previously reported QTL were located in the similar locus of the QTL identified in our study

QTL in rice (Table 3; Fig. 2). It has been reported that most QTL for domestication related traits are clustered in chromosomal blocks and the position of these clusters are consistent with those reported in different populations (Lee et al. 2005). Therefore, it is difficult to conclude whether the QTL identified in our study are the same QTL as those previously identified in other studies. Furthermore, to our knowledge, this is the first study to compare QTL of plant height and heading date for rice under both greenhouse and field conditions. This advancement is important because it may encourage researchers worldwide to identify useful QTL involved in plant height and heading date under controlled environmental conditions.

Many of the QTL reported by Yano et al. (1997) were located in regions similar to the heading date QTL identified in this study (Table 3; Fig. 2). The most effective heading date QTL, *qHD3.1*, accounted for 21 % of total phenotypic variation and was found near *Hd9* on chromosome 3 (Lin et al. 2002). As shown in Table 3, the map location of two other QTL, *qHD7.1* and *qHD7.2*, are similar to the location of *Hd2* and *Hd4* on chromosome 7 (Lin et al. 2000; Yano et al. 1997). *qHD8.1* was also detected near *Hd5* on chromosome 8 (Lin et al. 2002; Yano et al. 1997). *qHD8.2* was located near *qDTH-8* previously observed in an *Oryza glaberrima* by *Oryza sativa* cross in which the favorable allele came from *O. glaberrima* (Suh et al. 2005). Our results indicate that *O. sativa* alleles can possibly be used to reduce heading; however the magnitude of effect is not as high as that of observed in *O. glaberrima*. *qHD10.1* was located near *Ehd-1* (Doi et al. 2004). Additionally, the conserved chromosomal locations on heading date QTL may suggest that the QTL may have undergone human selection to diversify the heading date of rice during crop domestication or the early stage of cultivation. Understanding the molecular basis involved in this epistasis would be a foundational advancement in regulation of plant growth rate.

Additive-by-additive epistatic interaction was observed between *qHD7.1* and *qHD8.1*. The presence of both Zhe733 alleles can reduce heading date by 6 days. An epistatic interaction in this region has been observed in a cross of a weedy rice accession with a domesticated pure line of *O. sativa* (Gu and Foley 2007). This is the first time such an interaction was identified in a cross involving two domesticated *O. sativa* varieties. Molecular basis of epistatic interaction of these two QTL needs to be elucidated in the future. The development of target amplification polymorphism (TRAP) marker (Hu and Vick 2003) at *qHD7.1* and *qHD8.1* should help to delimit the genomic regions and shed more light on the molecular basis of epistatic interactions.

The sum of the additive effects of the plant height QTL identified in our study was similar to the differences in plant height between KBNT*lpa*1-1 (*japonica*) and Zhe733 (*indica*). However, as shown in Fig. 1, a large phenotypic variation of RIL individuals was observed in the frequency distributions of plant height. A number of RILs were much taller (>30 cm) than KBNT*lpa*1-1 (104 cm), indicating the complexity of genetic regulation of plant height. Even though two plant height QTL were identified between KBNT*lpa*1-1 and Zhe733, a single QTL locus may contain multiple alleles for plant height. In rice, it has been reported that dozens of genes play a role in regulating plant height (Sakamoto and Matsuoka 2004). Any possible combination of genes associated with plant height can directly affect metabolic pathways for plant development. Phenotypic gain without genetic contribution may suggest that transgressive variation observed for plant height may also be due to epigenetic regulation. Indeed, several nucleotide differences nearby height controlling gene, *OsBAK1*, were predicted to alter the gene's functions (Li et al. 2009). Continued examination of these changes that affect the binding of transcription factors resulting in altered gene expression should provide a better understanding of epigenetic regulation.

The two subspecies of cultivated rice, *japonica* and *indica*, have different evolutionary histories and their potentially independent domestication has resulted in distinctly different genomes (He et al. 2011). Pleiotropic effects on both heading date and plant height have been reported in the population of Lemont and Teqing (Li et al. 1995). All QTL for heading date in Lemont and Teqing populations were mapped to similar locations as plant height QTL. In the present study, heading date and plant height did not correlate with each other. Indeed changes in biochemical and physiological states can affect and promote gene expression profiles that are associated with plant height. Whether or not there are any pleiotropic effects on these two traits is difficult to predict, it is known that genes controlling heading date are associated with the transition of rice from the vegetative phase to the reproductive phase (Yan et al. 2011). To determine any pleiotropic roles in regulating multiple traits in rice, the gene needs to be cloned and functionally characterized.

qPHT1.1 and *qPHT3.1* are located in the same genomic region as other known QTL reported in previous studies (Table 3). Two other knownQTL, *Ph3* and *ph3-2*, are located close to *qPHT3.1* (Yan et al. 1998). The semi-dwarf gene, *sd-1*, of rice was found on chromosome 1 at the location of 38.7 Mb (Monna et al. 2002; Spielmeyer et al. 2002). One of the plant height QTL, *qPHT1.1*, lies near this location. The SSR marker, RM431, closely linked to *qPHT1.1*, was found on chromosome 1 at 39.2 Mb and this marker is 0.5 Mb away from *sd-1*. Zhe733 is semi-dwarf and the majority of semi-dwarf varieties contain *sd-1* (Spielmeyer et al. 2002). Zhe733's pedigree has IR24, which contains *sd-1* (Khush and Gomez 1985; Monna et al. 2002), indicating that *sd-1* may be one of the factors controlling height variations in Zhe733.

Marker assisted selection (MAS) is a powerful tool to accelerate conventional rice breeding. Despite abundant DNA markers for heading date, flowering time, and plant height reported in rice (Hittalmani et al. 2003; Yu et al. 2002; Septiningsih et al. 2003; Thomson et al. 2003; Yan et al. 1998; Yano et al. 2001; Moncada et al. 2001; You et al. 2006), few breeding programs heavily depended on the use of MAS for selection for traits other than disease resistance (Bertrand et al. 2008). Possible reasons to explain the low impact of MAS in rice improvement are (i) QTL effects vary depending on genetic background or are influenced by environmental conditions, (ii) accuracy of QTL mapping studies, and (iii) poor integration of molecular genetics and conventional breeding (Bertrand et al. 2008). Among these reasons, reliability of QTL mapping is the most critical step to the success of MAS.

For accurate QTL mapping, reliable phenotypic data using multiple replications, environments, and

confirmation of QTL results in independent populations are critical. QTL governing heading date and plant height identified in the present study are also conserved across many different mapping populations used by other research groups. The major QTL of these two traits can now be detected under controlled greenhouse conditions suggesting that major QTL have consistent effects across environmental conditions. This finding suggests that the major QTL may have been strongly selected during rice domestication. Nevertheless, SSR markers tagging QTL controlling heading date and plant height identified in our study should be an additional valuable molecular tool for breeding for early flowering semi-dwarf rice possibly resulting in substantial yield improvements.

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