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Mapping of quantitative trait loci for plant height and heading date in two inter-subspecific crosses of rice and comparison across *Oryza* genus

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ABSTRACT. Two inter-subspecific segregating populations were constructed to map quantitative trait loci (QTLs) for plant height (PH) and heading date (HD) in rice. A total of 99 and 95 polymorphic SSR and STS markers were used to establish linkage maps in *japonica* TK8 × *indica* IR1545-339 and *japonica* Nipponbare × IR1545-339, respectively. By employing composite interval mapping verified by single point analysis, ten PH and seven HD QTLs were located on seven and six chromosomes, respectively; only *Qph1.2* and *Qhd3* were common in the two populations. The phenotypic variance explained (PVE) by major PH and HD QTLs was 22.07% (*Qph6.1*) and 12.98% (*Qph6.2*), and 35.74% (*Qhd3*) and 15.54% (*Qhd3*), for TK8/IR1545-339 and Nipponbare/IR1545-339, respectively. Comparative genetic analysis of QTL intervals on rice pseudomolecules showing the convergence of QTLs affecting PH and HD across the *Oryza* genus revealed all QTLs uncovered in the two populations possibly allelic to QTLs previously published. By comparative mapping and candidate gene approach, we specified three PH, *Qph1.2*, *Qph4*, and *Qph6.1*, and one HD QTL, *Qhd10*, without high-resolution mapping, and the reality of QTLs identified by interval mapping were discussed herein.

Keywords: Comparative genetics; Candidate gene approach; Heading date; Plant height; Quantitative trait locus (QTL); Rice.

INTRODUCTION

Rice, domesticated 9000 years ago and grown from 55°N and 36°S, contains thousands of cultivated varieties belonging to two species, *Oryza sativa* grown mainly in Asia and *Oryza glaberrima* grown mainly in West Africa (Khush, 1997). Thus, rice, as the leading crop worldwide, provides 23% of the calories consumed by humans and feeds more than 50% of population. About 90% of rice is produced in Asia, and the demand for rice in Asia will increase by 40% by 2030 (Khush, 2005). Other than yield components, plant height (PH) and heading date (HD) are two important traits related to yield potential and are detrimental in rice domestication and modern breeding programs. PH plays an important role in yield improvement during breeding programs, as was shown in the most famous historical milestone with a semi-dwarf variety, IR8, invoking the "Green Revolution" in the late 1960s. Varieties with reduced height can avoid wind and rain damage for resistance to lodging and for increase in yield with adequate fertilization by nitrogen. HD, or days to flowering, is one of the critical traits for rice adaptation in diverse environments and rice cultivation in various regions and cropping seasons.

In general, plant height of rice is regulated by several genes and influenced by the environment. The dwarf genes, *d-1* to *d-60*, and semi-dwarf genes, *sd-1* to *sd-7*, were found to be induced artificially by radiation or chemicals or naturally identified; some were mapped by classical genetic analysis (Kinoshita, 1995). Dozens of PH genes were also detected in various interspecific, inter-subspecific and intra-subspecific crosses (Li et al., 1995; Xiao et al., 1996; Zhuang et al., 1997; Yan et al., 1998; Ishimaru et al., 2001; Yu et al., 2002; Li et al., 2003; You et al., 2006). Quantitative trait loci (QTLs) for PH, isolated to elucidate

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functions under molecular, biochemical and physiological levels, participate mostly in the metabolism and signal transduction of phytohormones. Deficiency in gibberellin acid (GA) and brassinosteroids, which can stimulate cell division and elongation, hinders plant growth and results in a dwarf stature. Examples include *D18, D35,* and *sd-1* involving a GA synthetic pathway (Sasaki et al., 2002; Itoh et al., 2004); *D1, SLR1, GID1* and *GID2* involving a GA signal transduction pathway (Ashikari et al., 1999; Sasaki et al., 2003; Ueguchi-Tanake et al., 2005); *D2* and *D11* involving a brassinosteroid synthetic pathway (Hong et al., 2003; Tanabe et al., 2005); and *D61* involving a brassinosteroid signal transduction pathway (Yamamuro et al., 2000). In addition, many genes involving cell division and elongation and development of apical meristem have great effect on PH. Recently, knowledge of the regulation mechanism of PH has been incorporated into breeding programs to generate short but high-yield rice (Ashikari et al., 2005).

In rice, HD, or flowering time, is controlled by both qualitative genes (i.e. photoperiodic and circadian genes) and quantitative genes of unknown functions and is influenced by conditions other than day length such as temperature and so on. A total of 15 QTLs affecting HD were detected by interval mapping of the F_2 population and several advanced backcross populations of *O. sativa* ssp. *japonica* Nipponbare × *O. sativa* ssp. *indica* Kasalath, five of these-*Hd1, Hd2, Hd3, Hd5,* and *Hd6*- were regulated by day length (Yano et al., 2001; Yamamoto et al., 2000). Numerous QTLs were also identified from several interspecific, inter-subspecific and intra-subspecific crosses (Kinoshita, 1995; Li et al., 1995; Zhou et al., 2001; Yu et al., 2002; Li et al., 2003; Thomson et al., 2003; Fujino et al., 2005; You et al., 2006). To date, some rice HD genes, isolated via positional cloning and gene functions explored at the molecular level, were found to be involved in (1) light-controlled photoperiodic response, such as *Hd6* encoding the α subunit of casein kinase II; *Hd3a*, functioning as florigen similar to *Arabidopsis FLOWERING LOCUS T* (*FT*); and *Ehd1*, encoding a B-type response regulator acting as a floral inducer under short day; or (2) clock-controlled circadian response, such as *Hd1*, which functions as a transcription similar to *CONSTANS* (*CO*), and *Hd2*, suspected as a pseudo response regulator (Yano et al., 2000; Takahashi et al., 2001; Kojima et al., 2002; Murakami et al., 2005). The function and structure of genes involved in flowering time in rice, a short-day plant, and in of *Arabidopsis*, a long-day plant revealed a conserved floral pathway but with minor difference, which the function of *Ehd1* is unique to rice (Izawa, 2010).

Allelic variations in HD and PH exist in various natural and bred varieties under natural or artificial selection pressure. QTLs are identified according to gene segregation in a segregating population, which is based on the allelic polymorphism between parental lines. Therefore, continuing to discover QTLs by using different pedigrees will reveal not only novel genes but also genes expressed consistently, which would be desirable for breeding purposes or for positional cloning for further investigation. Here we report on the identification of QTLs affecting PH and HD in two inter-subspecific crosses, *O. sativa* ssp. *japonica* cv TK8 × *O. sativa* ssp. *indica* IR1545-339 (TK8/IR1545-339), and *O. sativa* ssp. *japonica* cv Nipponbare × *O. sativa* ssp. *indica* IR1545-339 (Nipponbare/IR1545-339) and their comparison with conservative QTLs across the *Oryza* genus in public databases. In addition, we sequenced and aligned Nipponbare, TK8 and IR1545-339 alleles of three PH and two HD genes to confirm the conservative QTLs by comparative mapping.

MATERIALS AND METHODS

Plant materials

Two *O. sativa* ssp. *japonica* varieties, TK8, an elite cultivar in Taiwan, and Nipponbare, a popular cultivar in Japan and used for rice genome sequencing, were crossed to an *O. sativa* ssp. *indica* variety, IR1545-339, a bacterial blight-resistant variety developed by the International Rice Research Institute (IRRI, Los Baños, Philippines). A total of 304 and 301 F_2 individuals derived from TK8/IR1545-339 and Nipponbare/IR1545-339, respectively, were planted in the second crop season in 1999 for evaluating PH. Approximately 16 F_2-derived F_3 individuals for each F_2 plant were grown in the first crop season in 2000 for evaluating HD. All experimental populations were planted at the Taoyuan District Agriculture Research and Extension Station, Taoyuan, Taiwan.

Measurement of phenotypes

PH was measured from soil surface to the top of the panicle of the tallest tiller at maturity in centimeters. HD was from transplantation to the paddy field until complete exertion of one or more panicles with 50% of flowering spikelet, and the HD for each F_2 was estimated from the mean HD of approximately 16 F_3 progenies.

Molecular marker assay

Three grams of seeds of each F_2 individual, about 100 seeds which could represent genotype of F_2, were planted in pots for 3 to 4 weeks. The leaves of young seedlings were harvested, lyophilized, and then ground into fine powder for genomic DNA extraction. The procedures of DNA extraction followed Li et al. (1995) with minor modifications. About 0.5 gram of powdered leaf tissue was added to 9 ml of extraction buffer (100 mM Tris, pH 8.0; 50 mM EDTA, pH 8.0; 500 mM NaCl; 1.25% SDS; fresh-made 0.38% of $NaHSO_3$), mixed well, and incubated in a 65°C water bath for 1 h with occasional inversion of the mix. The extraction mixture was added to 2.7 ml of 5 M potassium acetate, gently mixed by inversion, and then set on ice for 20 min. Supernatants were collected after centrifugation at 3,500 rpm (TJ-25 Centrifuge, Beckman Coulter, USA) for 20 min at 65°C and were added to 10 ml of ice-cold isopropanol to precipitate DNA. DNA pel-

lets were hooked, soaked in 3 ml purifying buffer (70% of ethanol, 0.3 M sodium acetate) overnight, washed with cold 70% of ethanol, air dried, and then dissolved in 200 µl of TE (10 mM Tris, pH 8.0; 1 mM EDTA, pH 8.0).

Two types of PCR-based markers, SSRs and sequence tagged sites (STSs), previously mapped on the 12 rice chromosomes or discovered by bioinformatics (IRGSP, 2005), were used to establish linkage maps. Primers of SSR and STS markers were synthesized by GENSET (Singapore Biotech), and sequences are available from Gramene and the Rice Genome Research Program. The PCR reaction, containing 50 ng genomic DNA, 200 µM of each deoxynucleotide triphosphate (ABgene, UK), 0.2 µM of forward and of reverse primers, and 0.5 unit of *ProTaq* and 1× *Taq* buffer (Protech, Taiwan) in a final volume of 25 µl, was amplified by use of a thermocycler (Model T1, Biometra, Germany) at 94°C for 1 min for 1 cycle; 94°C for 1 min, 55°C for 1 min, 72°C for 2 min for 35 cycles; and 72°C for 5 min for 1 cycle. Three µl of amplified DNA products was separated on 2.5% of SFR agarose (Amresco®, USA) by using RAGE electrophoresis system (Rapid Agarose Gel Electrophoresis, Cascade Biologics, Oregon, USA) in 1 × TAE at 250 V for 9 to 23 min, depending on the size difference between amplified DNA fragments of SSR or STS alleles.

Analyses of linkage maps and QTLs affecting PH and HD

The linkage maps were constructed with 99 and 95 polymorphic markers for TK8/IR1545-339 and Nipponbare/IR1545-339, respectively. Genetic distance between pairs of markers was estimated by use of MAPMAKER/EXP, version 3.0 with the Kosambi function (Lander et al., 1987). The LOD threshold was fixed at 3.5 for unlinked markers, with genetic distance larger than 40 cM.

QTL analysis was employed by use of QTL Cartographer, version 2.0 (Wang et al., 2001). To investigate the association between phenotypic variation and marker genotype, we first used single point analysis (SPA) to detect the linkage of quantitative traits to markers individually by F-test at four significance levels: α= 0.05, 0.01, 0.001, and 0.0001. Then, composite interval mapping (CIM) was used to detect 90% confidence intervals of chromosomal regions of putative QTLs. Model 6, which uses a specified number of markers that fall outside a window around the flanking markers, was applied. A window size of 2 cM was used to scan the whole genome with forward stepwise regression. A LOD threshold of 2.4 was needed p = 0.05 for declaring the significance of a putative QTL. QTLs detected through CIM analysis were assigned with an abbreviation of the trait name (HD for heading date and PH for plant height) and the chromosome number, and numbers were affixed at the end if more than one locus was identified on the same chromosome. The phenotypic variance explained by the putative QTLs was estimated by the coefficient of determination (R^2), with maximum likelihood used for CIM (Basten et al., 2001).

Comparative analyses of QTLs affecting PH and HD

The QTLs detected in this study underwent comparative genetic analysis across the *Oryza* genus. The complete sequencing of the rice genome allows for identifying homology on the basis of overlapped intervals along physical maps (IRGSP, 2005). We performed a BLAST search of TIGR Rice Genome Pseudomolecules V.4 with the primer sequences of SSR and STS markers flanking QTLs and sequences of cloned genes for PH and HD to delineate physical chromosome segments of QTL intervals. In addition, physical positions of some published QTLs and markers are available from Gramene. Only physical intervals of QTLs overlapping with the identified QTLs in our studies were considered homology.

After comparative analysis by physical mapping, we identified numerous published PH and HD QTLs corresponding to our discovery. Three PH related genes, *Sd1* (accession no. AF465255), *D35* (accession no. AK287607), and *OsKS1* (accession no. AB126933), and two HD genes, *Ehd1* (accession no. AB092506) and *Hd1* (accession no. AB041838), were subjected to analyze the alleles of IR1545-339 and TK8 to reveal the reality of QTL mapping and comparative QTL mapping. The recessive allele of *sd1* is well-known as 383-bp deletion (Sasaki et al., 2002), the primer sequences flanking the deletion were designed, sd1-del-F: 5'-CACAgCgCTCACTTCTCATC-3' and sd1-del-R: 5'-CTTACATggCgTCgTCACAC-3'. For the other four genes, primer walking based on the published gene sequences of Nipponbare were employed to accomplish those gene sequences of IR1545-339 and TK8.

RESULTS

Genetic variation for plant height and heading date

The PH of the tallest tiller of parents, TK8, Nipponbare, and IR1545-339 was 108, 90, and 100 cm, respectively. The mean PH was 108.3 (±12.7) cm (range 73 to 141 cm) and 101.0 (±12.2) cm (range 70 to 132 cm) for the F_2 populations of TK8/IR1545-339 and Nipponbare/IR1545-339, respectively (Figure 1). The male parent IR1545-339 was shorter than the female parent TK8 by 8 cm but was taller than the female parent Nipponbare by about 10 cm, and transgressant progenies were found in both F_2 populations of the two pedigrees (Figure 1). The phenotypic segregations in these two F_2 populations exhibited normal distribution, a typical phenomena of quantitative a trait, which indicates that PH was regulated by several genes and influenced by the environment.

Days to heading, the date of exertion of at least one panicle with 50% of the flowering spikelet, of the parents TK8, Nipponbare, and IR1545-339 were 93, 69, and 103 days, respectively (Figure 1). Mean HD of approximately 16 F_3 progenies for each F_2 individual was 95.6 (±5.3) days (range 80 to 122 days) for TK8/IR1545-339 and 84 (±4.5) days (range 72 to 99 days) for Nipponbare/IR1545-339. The male parent IR1545-339 showed delayed heading, by approximately 10 and 34 days, as compared with TK8

and Nipponbare, respectively, and transgressant progenies were observed only in TK8/IR1545-339 and not in Nipponbare/IR1545-339. The frequency distributions for HD in these two crosses were normal (Figure 1), which indicates that HD was a polygenic inheritance.

Linkage maps

A total of 99 polymorphic markers, 88 SSR and 11 STS, comprising 90% of the rice genome as referred to the established map published by IRGSP (http://rgp.dna.affrc.go.jp/E/IRGSP/index.html), were employed in the 304 F_2 progenies of TK8/IR1545-339. Twelve linkage groups spanning 1342.9 cM, with an average distance of 16.65 cM, were established (Figure 2). The linkage map of Nipponbare/IR1545-339 was composed of 95 polymorphic markers, 75 SSR and 20 STS, applied to 301 F_2 progenies. The map included 12 linkage groups spanning 1027.4 cM, with an average distance of 10.81 cM, and covered 85% of the rice genome. All marker orders of these two linkage maps agreed with the physical maps obtained by BLAST search against rice genome sequences (IRGSP, 2005), except three intervals of *RM1387/RM104*, *RM1367/RM263*, and *R20049/RM16* located on chromosomes 1, 2 and 3, respectively.

A total of 117 polymorphic markers were applied in these two F_2 populations for QTL analyses. Seventy-seven (66%) markers were commonly polymorphic between TK8/IR1545-339 and Nipponbare/IR1545-339 and could serve as anchor markers to align two linkage maps for further comparative analysis of QTL map position. However, 22 and 18 markers were specifically polymorphic to TK8/IR1545-339 and Nipponbare/IR1545-339 and are indicated with superscripts T and N, respectively (Figure 2). The differential marker polymorphism revealed allelic variation among TK8, Nipponbare, and IR1545-339, which implied various alleles of QTLs affecting PH and HD and different QTLs with some common QTLs would be detected in these two crosses.

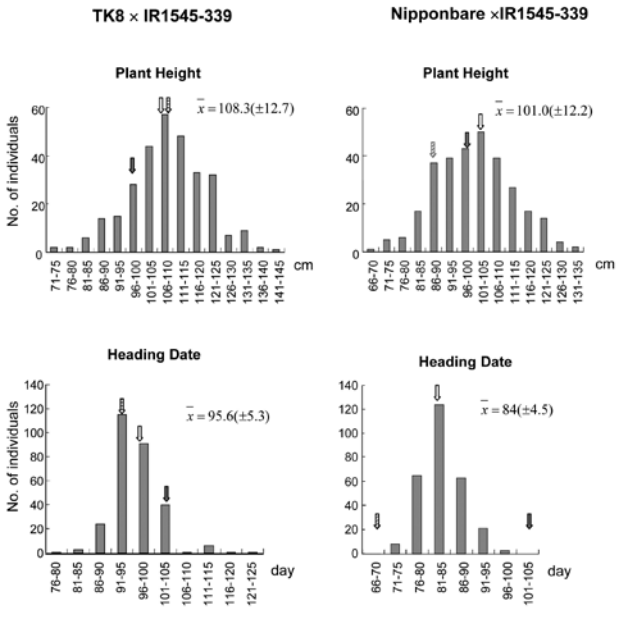

Figure 1. Frequency distributions for plant height and heading date in segregating populations derived from the intra-subspecies crosses between *japonica* TK8 × *indica* IR1545-339 and *japonica* Nipponbare × *indica* IR1545-339. The arrows indicate the intervals of trait values of parents TK8 (⇨), Nipponbare (⇨), and IR1545-339 (⇨), and of F_2 (⇨). The numbers in parentheses represent the standard deviation for plant height and heading date.

QTL analyses

We employed SPA and CIM to detect segregating QTLs affecting PH and HD. Large QTLs with more phenotypic variance explained (PVE) effects deduced from CIM analysis usually show significance. However, two of ten PH QTLs, *Qph4* and *Qph11*, and one of seven HD QTLs, *Qhd9*, did not show significance by SPA; these three QTLs were detected by relatively small LOD and contributed less PVE (Table 1). This discrepancy of QTL results is commonly found in several QTL studies involving SPA, interval mapping (IM), CIM, and multiple interval mapping (MIM) because of different algorithms and statistical methods used. Since interval mapping involves the use of genotype information of two markers simultaneously rather than one marker a time, we used PH and HD QTLs with intervals of significant LOD after CIM analysis.

Mapping QTLs for plant height

Four QTLs affecting PH were identified in TK8/IR1545-339 (Figure 2), with PVE ranging from 4.17% to 22.07% (Table 1). Two of the four, *Qph1.2* and *Qph6.1*, contributed large PVE of 19.16% and 22.07%, respectively, and the other two, *Qph2.1* and *Qph9*, contributed small PVE of 4.17% and 5.28%, respectively. All four showed partial dominance. The three alleles from TK8, $Qph1.2^{TK}$, $Qph2.1^{TK}$, and $Qph9^{TK}$, increased PH by 9.63, 4.08, and 4.05 cm, respectively. The allele $Qph6.1^{IR}$, in contrast, increased PH by 8.38 cm (Table 1).

Seven PH QTLs were detected in Nipponbare/IR1545-339, with PVE ranging from 3.28% to 12.98% (Table 1). Two of seven QTLs contributed PVE greater than 12% and the other five QTLs contributed PVE less than 5%. The Nipponbare alleles of four PH QTLs, $Qph1.1^{NB}$, $Qph1.2^{NB}$, $Qph4^{NB}$, and $Qph11^{NB}$, provided increased height, and the IR1545-339 alleles for the other three QTLs, $Qph2.2^{IR}$, $Qph5^{IR}$, and $Qph6.2^{IR}$, provided increased height. These three QTLs displayed an overdominant manner, with the other four an additive manner.

We uncovered 10 QTLs affecting PH, including one mapped on chromosome 1, with flanking markers *E60551* and *RM1387* identified in both populations. In addition, the interval for *Qph6.1* identified in TK8/IR1545-39 was mapped between *R3879* and *RM30* on chromosome 6, which is the next adjacent interval to *Qph6.2*, *RM30* and *RM340*. *Qph6.1* and *Qph6.2* could belong to the same PH gene because of unresolved mapping with the genotyping

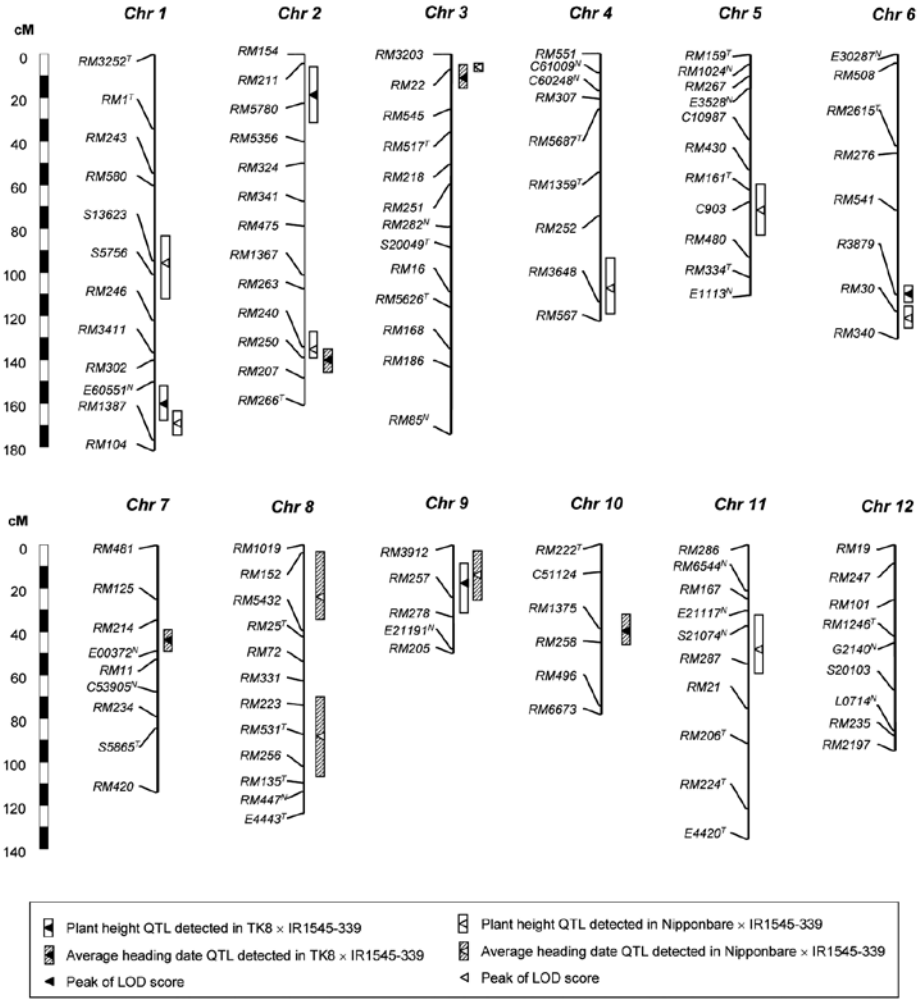

Figure 2. QTLs for plant height and heading date along the genetic linkage map of two F_2 populations derived from TK8/IR1545-339 and Nipponbare/IR1545-339. The 90% of confidence intervals of QTLs is plotted with the LOD score peak indicated by a *solid triangle*.

and phenotyping data analyzed by CIM. However, these two QTLs mapped on chromosome 6 identified in these two populations independently contributed the largest PVE with incongruity (Table 1).

Mapping QTLs for heading date

Four QTLs affecting HD were identified in TK8/IR1545-339 (Figure 2), with PVE ranging from 3.46% to 35.74% (Table 1). The largest, *Qhd3*, was mapped between *RM3203* and *RM545* on chromosome 3 and contributed up to 35.74% of the PVE. The QTLs, *Qhd7* and *Qhd10*, also had large effects on HD, with 12.66% and 12.91% of PVE, respectively (Table 1). Two TK8 alleles, $Qhd3^{TK}$ and $Qhd7^{TK}$, and two IR1545-339 alleles, $Qhd2^{IR}$ and $Qhd10^{IR}$, promoted heading in the first crop season in 2000; the two TK8 alleles promoted heading, for a total of approximately 9 days, whereas the IR1545-339 alleles promoted heading for three days only. One of the four QTLs, *Qhd2*, exhibited significant underdominance, but the other three acted as partial dominance in TK8/IR1545-339.

We identified four QTLs affecting HD in Nipponbare/IR1545-339, with PVE ranging from 5.06% to 15.54%. The largest, *Qhd3*, mapped between *RM3203* and *RM545* on chromosome 3, promoted heading and explained 15.54% of the PVE with an additive effect of 4.58 days and dominant deviation of 1.5 days. However, two alleles, $Qhd8.2^{NB}$ and $Qhd9^{NB}$, postponed heading for about two days each (Table 1). Three QTLs, *Qhd1*, *Qhd8.2*, and *Qhd9*, performed as partial dominance and one, *Qhd8.1*, performed as overdominance. The QTL intervals of *Qhd8.1* and *Qhd8.2* did not overlap, and gene actions and allele contributions differed, which supports that the two HD QTLs were detected on chromosome 8.

We uncovered seven QTLs affecting HD. *Qhd3* contributed the largest PVE of HD, and $Qhd3^{IR}$ delayed flowering for approximately five days in both crosses with incongruity.

Table 1. Parameters of QTLs for plant height and heading date detected in the crosses of TK8 × IR1545-339 and Nipponbare × IR1545-339.

Trait	Chr	QTLs	Flanking markers[a]	*TK8 × IR1545-339*					*Nipponbare × IR1545-339*				
				LOD	a^b	*d*	d/[a][b]	PVE%[c]	LOD	a^b	*d*	d/[a][b]	PVE%[c]
Plant Height	1	*Qph1.1*	*RM580 - RM246***						2.64	4.73	0.57	0.12	3.28
	1	*Qph1.2*	*E60551 - RM1387****	7.84	9.63	3.51	0.36	19.16	7.96	6.46	-2.22	-0.34	12.86
	2	*Qph2.1*	*RM211 - RM5356**	3.03	4.08	2.05	0.50	4.17					
	2	*Qph2.2*	*RM263 - RM250****						3.38	-0.74	4.83	6.52	4.78
	4	*Qph4*	*RM252 - RM567*^{NS}						2.96	6.36	3.02	0.47	4.64
	5	*Qph5*	*RM430 - RM480****						2.80	-1.97	2.89	1.47	3.87
	6	*Qph6.1*	*RM541 - RM30****	16.49	-8.38	1.12	0.13	22.07					
	6	*Qph6.2*	*RM3879 - RM340****						9.34	-2.32	6.77	2.92	12.98
	9	*Qph9*	*RM3912 - RM278**	3.11	4.05	2.91	0.72	5.28					
	11	*Qph11*	*E21117 - RM21*^{NS}						2.42	5.42	2.14	0.39	3.94
Heading Date	2	*Qhd2*	*RM240 -RM207**	3.29	0.19	-2.00	-10.26	3.46					
	3	*Qhd3*	*RM3203 - RM545****	23.47	-5.21	-3.01	-0.58	35.74	10.62	-4.58	-1.50	-0.33	15.54
	7	*Qhd7*	*RM214 - RM11**	5.68	-3.61	-2.65	-0.73	12.66					
	8	*Qhd8.1*	*RM1019 - RM5432****						3.50	-0.40	2.27	5.67	7.89
	8	*Qhd8.2*	*RM331 - RM447***						2.89	2.35	1.30	0.55	5.06
	9	*Qhd9*	*RM3912 - RM278*^{NS}						2.70	2.44	1.84	0.76	5.39
	10	*Qhd10*	*C51124 - RM258***	9.46	2.16	-2.00	-0.92	12.91					

[a] The significance levels of nearest markers detected by SPA; NS, *, **, and *** indicate nonsignificant, $p<0.05$, 0.001, and 0.0001, respectively.
[b] The direction of additive effects was from either TK8 or Nipponbare in the two population. d/[a] indicates degree of dominance.
[c] Phenotypic variance explained by individual QTL.

Comparative QTL analyses of plant height and heading date

By QTL convergence comparison, we found 10 PH QTLs potentially homologous to other QTLs by significant overlap of intervals, and none were novel. Half of the 10 PH QTLs we identified corresponded to three dwarf genes, *d30*, *d57*, *d27*, and two semi-dwarf genes, *sd-1* and *d35*. The chromosome intervals of PH QTLs corresponding to *Sd-1* were identified from at least eight pedigrees besides our studies (Table 2). In our studies, one PH QTL, *Qph1.2*, corresponding to *Sd-1,* was identified in both populations, with a main contribution to PVE (Table 1). *Qph6.1*, mapped in TK8/IR1545-339 only, was corresponded to another isolated PH gene is *D35*, encoding *ent*-Kaurene oxidase, and the recessive allele *d35* is defective in GA synthesis (Itoh et al., 2004). The intervals of *Qph11*, corresponding to *d27* and *Qph4* encompass *OsKS8* and *OsKS1*, respectively, which both encode *ent*-Kaurene synthase, catalyzing the early step in GA biosynthetic pathway. Additionally, the intervals of *Qph1.1* and *Qph5* encompass *OsGA2ox2* and *OsGA20ox4*, respectively, which both encode GA 2- and GA 20-oxidase, catalyzing the later steps in the GA biosynthetic pathway (Sakamoto et al., 2004).

Positional cloning and sequencing of *Sd-1* alleles revealed that a recessive allele of miracle rice IR8, derived from a native Taiwanese variety, Dee-geo-woo-gen, had a 383-bp deletion, which led a mutation of GA 20-oxidase (Sasaki et al., 2002). By using the primers flanking the deletion, the amplicons of IR1545-339 was truncated but TK8 and Nipponbare were in full-length (Figure 3). As the evidence, *Qph1.2*, identified in both populations, was because of segregation of *Sd-1*. After annotation of *D35* sequences, one amino acid substitution was found in exon III, which isoleucine in IR1545-339 but valine in TK8 and Nipponbare. However, *Qph6.1* was only found in TK8/IR1545-339 but not in Nipponbare/IR1545-339 (Table 2, Figure 3). It was possible that the adjacent PH QTL with largest effect, *Qph6.2*, impeded the identification of *D35* segregation in Nipponbare/IR1545-339. The sequence alignment of *OsKS1* showed no polymorphism at the coding region among three parents. Nevertheless, Nipponbare possessed different allele to IR1545-339 and TK8 at upstream of 5'-UTR region with 23 SNPs (Figure 3), which was presumed to influence gene expression of *OsKS1* leading to plant height variation because of these two alleles segregating in the F_2 population of Nipponbare/IR1545-339 (Table 2, Figure 2).

Our comparative analysis with HD QTLs detected by independent research groups allowed us to speculate on the function or effects of QTLs we identified herein. On searching chromosome segments of rice pseudomolecules of these seven HD QTLs for possible orthologous QTLs from other pedigrees, only *Qhd3*, segregated in TK8/IR1545-339 and Nipponbare/IR1545-339, corresponded to at least two QTLs, including *Hd9*. *Qhd10* might be allelic to *Hd14, dth10.1,* and *Ehd1*, on the basis of the physical interval of *C51124* and *RM258*. We also found possible allelic QTLs identified from previous studies to five HD QTLs, *Qhd2, Qhd7, Qhd8.1, Qhd8.2,* and *Qhd9* (Table 3).

Table 2. The convergence of QTLs affecting plant height identified in this study to those identified in previous studies.

Chr	QTLs	Flanking markers	Physical position[a]	Cross design[b]	Corresponding QTLs or nearest marker	Reference
1	*Qph1.1*	*RM580 - RM246*	9,603,452 ~ 27,663,181	N	*Ph1*	Xiao et al., 1996
					Qph1	Li et al., 2003
					Ph1.1	Septiningsih et al., 2003
					Ph1.1	Thomson et al., 2003
					Qph1b	Mei et al., 2005
1	*Qph1.2*	*E60551 - RM1387*	35,012,879 ~ 40,534,591	T, N	*Sd-1*	Xiao et al., 1992
					Ph1	Yan et al., 1998
					qPH1	He et al., 2001
					R2414	Ishimaru et al., 2001
					Ph1.1, ph1.2	Moncada et al., 2001
					qPHT-1	Hittalmani et al., 2002
					Ph1.1,	Septiningsih et al., 2003
					Ph1.2	Thomson et al., 2003
					Qph1a	Mei et al., 2005
2	*Qph2.1*	*RM211 - RM5356*	2,020,685 ~ 9,432,636	T	*d30*	Xiao et al., 1992
					Ph2	Xiao et al., 1996
2	*Qph2.2*	*RM263 - RM250*	25,865,402 ~ 32,774,538	N	*Ph2*	You et al., 2006
4	*Qph4*	*RM252 - RM567*	28182082 ~ 34,500,023	N	*Ph4*	Zhuang et al., 1997
					Ph4.1	Moncada et al., 2001
					QPh4c	Li et al., 2003
5	*Qph5*	*RM430 - RM480*	18,670,780 ~ 27,292,849	N	*Ph5*	Xiao et al., 1996
					Ph5-2	Yan et al., 1998
					QPh5	Li et al., 2003
					Ph5.1	Thomson et al., 2003
					Ph5	You et al., 2006
6	*Qph6.1*	*RM541 - RM30*	19,513,541 ~ 27,252,381	T	*D35*	Itoh et al., 2004
					Ph6	Xiao et al., 1996
					Ph6.1	Thomson et al., 2003
6	*Qph6.2*	*R3879 - RM340*	25,926,402 ~ 28,599,297	N	*Ph6*	Xiao et al., 1996
					Qph6	Mei et al., 2003
9	*Qph9*	*R3912 - RM278*	10,826,231 ~ 27,062,430	T	*d57*	Kinoshita, 1995
					QPh9a	Li et al., 2003
					Ph9b	You et al., 2006
11	*Qph11*	*E21117 - RM21*	4,553,531 ~ 23,239,202	N	*d27*	Abenes et al., 1994
					Ph11a	You et al., 2006

[a]The physical map of QTLs are based on TIGR rice genome pseudomolecules V.4
[b]T and N of the cross design are indicated TK8/IR1545-339 and Nipponbare/IR1545-339, respectively.

The function study of *Ehd1*, a photoperiodic gene regulating the transition of vegetative stage to reproductive stage, revealed that the mutation of glycine substituted by arginine in GARP domain was photoperiod insensitive under short day condition (Doi et al., 2004). After annotation analysis of *Ehd*1 sequences, both IR1545-339 and Nipponbare possessed the same wild type allele but TK8 possessed the mutated allele. Thus, *Qhd10* was only identified in TK8/IR1545-339 (Table 1, Figure 2). *Hd1*, mapped on chromosome 6, is another rice photoperiodic gene corresponding to *CONSTANS* in Arabidopsis (Yano et al., 2000). Three different alleles were identified that Nipponbare had the full length of wild type allele, but IR1545-339 and TK8 had four and two-bp deletion at the exon 2, respectively (Figure 3). No QTL was uncovered in Nipponbare/IR1545-339, which was discordant to the expectation of the segregation of function alleles of Nipponbare and null-function of IR1545-339. Nevertheless, it was consistent to the no QTL detected in TK8/IR1545-339 because of null function by deletion.

DISCUSSION

Assessments of PH and HD QTLs in TK8/IR1545-339 and Nipponbare/IR1545-339

Quantitative traits such as plant height and flowering time are regulated by multiple genes, environments, and interactions between genes (epistasis) and between genes and environments (G×E). Nevertheless, in specific crosses, QTLs can be detected as segregating loci only, not nonsegregating loci, even loci involved in regulating morphology. More QTLs can be identified by using various cross combinations of elite cultivars, wild accessions, and relative species. In this study, we crossed two elite *japonica* cultivars, TK8 and Nipponbare, to a wild *indica* accession IR1545-339, and used SPA and CIM analysis

Table 3. The convergence of QTLs affecting heading date identified in this study to those identified in previous studies.

Chr	QTLs	Flanking markers	Physical position[a]	Cross design[b]	Corresponding QTLs or nearest marker	Reference
2	*Qhd2*	*RM240 - RM207*	31,497,147 ~ 35,369,659	T, N	*C560*	Lin et al., 1998
					Hd7	Yamamoto et al., 2000
					C560	Ishimaru et al., 2001
					dth2.1	Moncada et al., 2001
					dth2.2	Septiningsih et al., 2003
					QHd2b	Li et al., 2003
3	*Qhd3*	*RM3203 - RM22*	776,123 ~ 1,499,104	T, N	*QHd3*	Mei et al., 2003
					Hd9	Yano et al., 2001
					QHd3a	Mei et al., 2005
7	*Qhd7*	*RM214 - RM11*	12,782,791 ~ 19,256,338	T	*Hd4*	Yano et al., 1997
					dth7.1	Moncada et al., 2001
					dth7.1	Thomson et al., 2003
					qDTH-7-1	Fujino et al., 2005
					hd7	You et al., 2006
8	*Qhd8.1*	*RM1019 - RM5432*	195,984 ~ 4,371,994	N	*C166*	Wang et al., 2002
					qDTH8	Miyata et al., 2007
8	*Qhd8.2*	*RM331 - RM447*	12,288,944 ~ 26,416,973	N	*QHd8a*	Li et al., 2003
					QHd8	Mei et al., 2005
9	*Qhd9*	*RM3912 - RM278*	10,826,231 ~ 27,062,430	N	*FTLQ3*	Sarma et al., 1998
					QTL9	Zhou et al., 2001
					QHd9	Li et al., 2003
10	*Qhd10*	*C51124 - RM258*	7,180,112 ~ 17,756,238	T	*Hd14*	Yano et al., 2001
					dth10.1	Thomson et al., 2003
					Ehd1	Doi et al., 2004

[a]The physical map of QTLs are based on TIGR rice genome pseudomolecules V.4
[b]T and N of the cross design are indicated TK8/IR1545-339 and Nipponbare/IR1545-339, respectively.

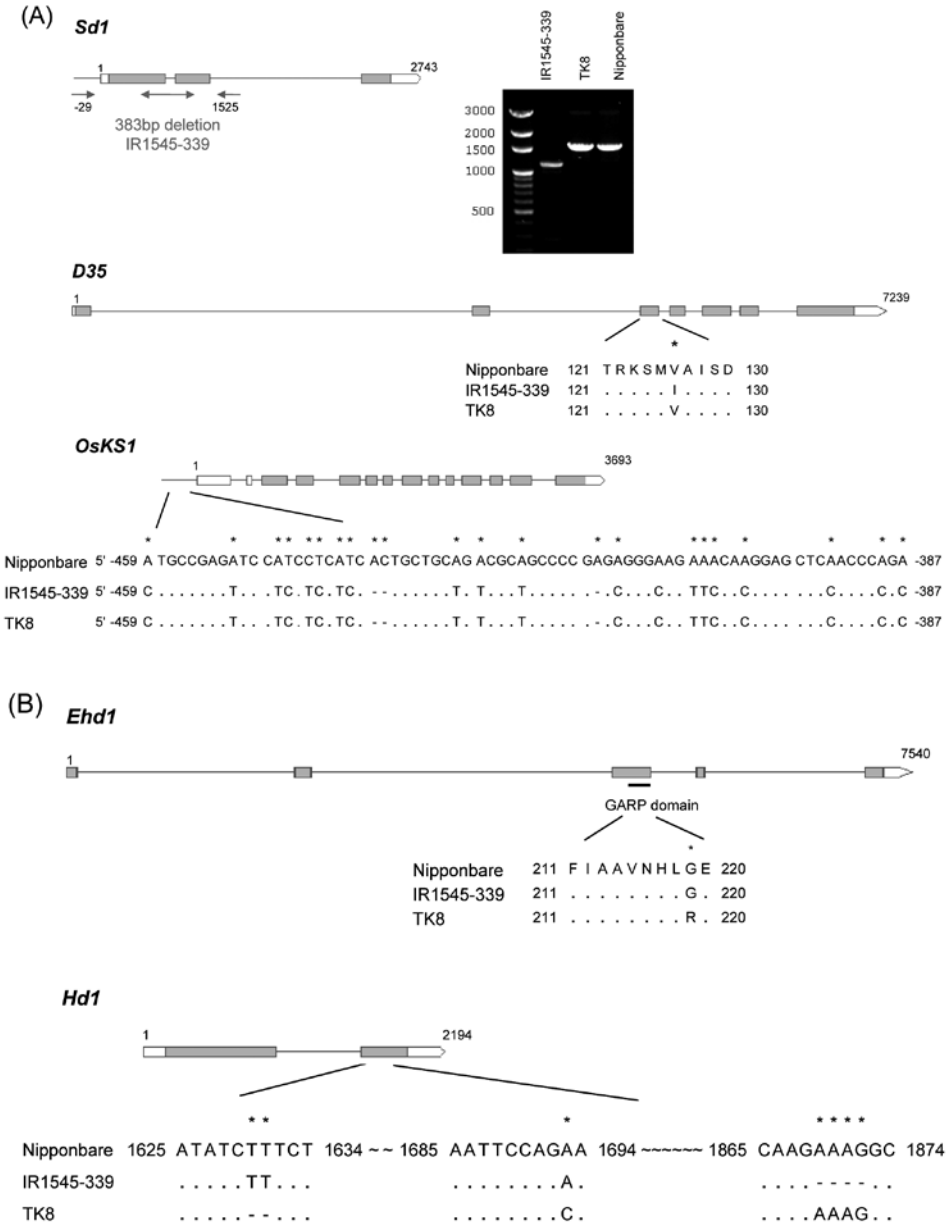

Figure 3. The alleles of three plant height genes (A) and two heading date genes (B) among Nipponbare, IR1545-339, and TK8. From the comparative analysis, the plant height genes, *Sd-1*, *D35*, and *OsKS1*, were corresponding to *Qph1.2*, *Qph6.1*, and *Qph4*, while the heading date gene, *Ehd1*, was corresponding to *Qhd10*. The * above nucleotide or amino acid indicates variation.

to identify PH and HD QTLs. In both TK8/IR1545-339 and Nipponbare/IR1545-339, only 66% of 117 markers were commonly polymorphic, and nearly all markers displayed three alleles among three parents. In addition, 22 and 18 specific polymorphic markers were employed independently to each cross, respectively. Various allelic polymorphisms in the two crosses implied various QTLs segregating in the F_2 populations, which can be detected by phenotyping, genotyping, and linkage analysis. Only one PH QTL, *Qph1.2*, and one HD QTL, *Qhd3*, were identified in both populations, and nine PH and six HD QTLs were unique to either population (Table 1). Thus, using more pedigrees of various parent combinations can help uncover more QTLs.

The phenotypic frequency distribution of quantitative traits is continuous with transgressive progenies whose phenotypes are beyond the phenotypes of parents. Most identified QTLs in this study performed as partial dominance, except for four PH QTLs in Nipponbare/IR1545-339 and one HD QTL in both populations (Table 1). The major reason for transgressive segregation could be complementary gene action for QTLs with opposite effects of parents (Thomson et al., 2003; Yano et al., 1997). In our study, the IR1545-339 alleles of PH and HD QTLs could either increase or decrease PH and HD in both F_2 populations, which is consistent with the above hypothesis.

The PVE of PH QTLs, four in TK8/IR1545-339 and seven in Nipponbare/IR1545-339, ranged from 4.17% to 22.07% and 3.28% to 12.98%, respectively. The phenotypic variation explained by the four HD QTLs in TK8/IR1545-339 and Nipponbare/IR1545-339 ranged from 3.46% to 35.74% and from 5.06% to 15.54%, respectively. More QTLs might have segregated in these two F_2 populations but were unable to be detected because of environmental or G×E influences, masking by QTLs with large effects, or resolution of statistical analysis. Use of advanced backcross designs by fixing QTLs with large effects could detect more QTLs with minor effects. From our study, no HD QTL corresponding to *Hd1* was detected Nipponbare/IR1545-339 even if the functional alleles of Nipponbare and null function of IR1545-339 should segregate in the F_2 population (Figure 3). Both *Hd1* and *Ehd1*, regulating the mobile florigen *Hd3a* expression, are involved in the photoperiod control of flowering in rice and promote heading under short day condition (Izawa, 2010). From the study of heading genes from three segregating population of BC_3F_2 derived from crosses of donor parent Tainung 67 and recurrent parent Koshihikari, *Ehd1* could explain 21.6~36.8% of PVE but *Hd1* could explain 2.5~6.5% only (Y.-R. Lin et al. unpublished data). The plausible possibility that the small effect of *Hd1* was masked by *Ehd1* for detection since the natural day length less than 13.5 hours in Taiwan is short day for heading. Because of adjacent to *Qph6.2*, *Qph6.1*, corresponding to *D35* was only found in TK8/IR1545-339 but not in Nipponbare/IR1545-339 even the same allele of TK8 and Nipponbare (Table 2, Figure 3). By candidate gene approach, we identified three PH and one HD gene without fine mapping and validate the reality of QTLs by interval mapping in agreement with the proposals of confirmation (Price, 2006; Yamamoto et al., 2009). Nevertheless, we cannot rule out the possibility that the phenotypic variation was caused by other genes resided in the same QTL intervals of 5~10 Mb. For example, *RFT1* and *Hd3a* are essential for rice flowering and are 11.5 kb apart chromosome 6 (Komiya et al., 2008). Even if there are some variations of DNA and amino acid after comparison, gene expression of the candidate genes for the three PH and one HD gene could provide further confirmation in three parental varieties.

In the grass family Poaceae, flowering always terminates vegetative growth, which results in a highly significant relation between plant height and flowering time. However, we found nearly no phenotypic correlation between PH and HD in TK8/IR1545-339 and Nipponbare/IR1545-339 (Spearman's rho = 0.01 and = 0.11, respectively) and no overlapped or closely linked QTLs affecting PH and HD from CIM analysis. Even though all QTLs, ten PH and seven HD, were uncovered independently in these two crosses, only two QTL intervals overlapped. Phenotypic correlation and gene identification analyses demonstrated no correlation between PH and HD in the two pedigrees.

Convergence of PH and HD QTLs across the *Oryza* genus

QTLs affecting PH and HD in rice have been extensively mined by linkage analyses of classical morphologies and molecular markers. A total of 1011 PH and 618 HD QTLs, annotated by Gramene in 2008, were published between 1994 and 2006 (Yamamoto et al., 2009). Four and seven QTLs affecting PH were identified in TK8/IR1545-339 and Nipponbare/IR1545-339, respectively (Table 1, Figure 2). By QTL convergence comparison, all 10 PH QTLs identified in this study showed potentially homologous to other published QTLs. The convergence of QTLs identified across varieties and species provide additional confirmation of QTL existence and also indicate the main contributions against evolution or artificial selection during breeding, and might be worth cloning for further investigation of biological function and application in rice breeding (Price, 2006; Yamamoto et al., 2009).

PH is significantly correlated with grain yield, so reduction in height could improve lodging resistance and harvest index. *Qph1.2*, identified in both populations with a main contribution to PVE, was corresponding to *Sd-1* which also uncovered from at least eight pedigrees (Tables 1, 2). The amplicons of *Sd-1* revealed *Sd-1* alleles, $Qph1.2^{TK}$ and $Qph1.2^{NB}$, were dominant and increased plant height by 9.63 and 6.46 cm, respectively, and $Qph1.2^{IR}$ was recessive resulting in reduced PH (Table 1, Figure 3). Three other dwarfing alleles encoding proteins with one amino acid substitution also arose from natural mutation or from gamma-ray (Sasaki et al., 2002; Spielmeyer et al., 2002). Other research groups have mapped PH QTLs corresponding to *Sd-1* (Table 2); the *sd-1* alleles from these studies might be the same four alleles mentioned above and be introgressed to these varieties of QTL mapping from the same lineage, or be different alleles. Even the recessive allele with 383-bp deletion of the *Sd-1* locus originating from a native Taiwanese variety, 'Dee-geo-woo-gen', none popular semi-dwarf varieties were derived from this *sd-1* allele in Taiwan. However, selecting different *sd-1* alleles for a desired effect on PH had been incorporated into rice breeding programs for producing ideotype varieties (Asano et al., 2007).

HD is one of critical characters for rice adaptation in various environments, and one of pivotal characters for rice breeding because of its major association with grain filling, yield, and quality. Two major factors affecting HD are basic vegetative growth (BVG) and photoperiod sensitivity, of which day length is a key environmental factor for triggering photoperiod-sensitive varieties to flower in rice. Photoperiod-insensitive cultivars such as TK8, Tainung67 (TNG67), and Taichung65 (TC65) were developed for allowing two crops a year in Taiwan, but Nipponbare is a photoperiod-sensitive cultivar in Japan. From sequenced and function-identified photoperiodic genes, alleles of TC65, TK8, and TNG67 for *Hd1* and *Ehd1* possessing loss of function led these Taiwanese cultivars into photoperiod insensitivity (Doi et al., 2004; Chen at al.,

2010). *Ehd1* has major effect on BVG that wild type allele promote heading under short day condition. The recessive *ehd1* allele of one amino substitution (G→R) of GARP domain was unique to TC65 and other Taiwan modern cultivars (Figure 3; Saito et al., 2009). This explained why *Qhd10* was only identified in TK8/IR1545-339 but not in Nipponbare/IR1545-339 (Table 1, Figure 2). Nevertheless, environmental factors other than day length, such as temperature and gene interaction (Luan et al., 2009), might account in part for failure of detection *Hd1* gene in Nipponbare/IR1545-339.

The QTLs identified by interval mapping and then by comparative mapping for QTL convergence in this study provide additional information about the natural variation of PH and HD QTLs during evolution and breeding. Through the candidate gene approach, we isolated four conserved QTLs without high-resolution mapping. QTLs conserved across many pedigrees might be worth isolating by candidate gene approach for further investigation and applied to breeding programs. The novel HD QTL we found might be enriched our understanding about flowering time in rice.

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水稻株高和抽穗期數量性狀基因座於兩個亞種雜交族群之圖譜分析及稻屬間之比較

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本研究建構兩個水稻亞種間的雜交族群，用於株高與抽穗期之數量性狀基因座圖譜分析，分別以 99 個和 95 個多型性的 SSR 和 STS 分子標幟於粳稻台粳 TK8 × 秈稻 IR1545-339 和粳稻日本晴 × IR1545-339 建立遺傳連鎖圖譜，經由組合區間定位法分析和單點分析。於七條和六條染色體上偵測出十個株高和七個抽穗期數量性狀基因座，其中只有 *Qph1.2* 是共通的，主要株高基因座於 TK8/IR1545-339 和 Nipponbare/IR1545-339 分別可解釋 22.07% (*Qph6.1*) 和 12.98% (*Qph6.2*) 之株高變異，而主要抽穗期基因座 *Qhd3* 則可分別解釋 35.74% 和 15.54% 之抽穗日數變異。經由於染色體偽分子之物理圖譜進行稻屬間之株高與抽穗期數量性狀基因座之比較分析，本研究發現數量性狀基因座皆與目前已發表之數量性狀基因座具有保守性，隨後利用比較數量性狀基因座的結果與候選基因策略，在未進行高解析之圖譜分析而成功的達到三個株高基因座 (*Qph1.2*, *Qph4*, and *Qph6.1*) 和一個抽穗期基因座 (*Qhd10*) 之確認，本文並討論區間定位法之正確性。

關鍵詞：比較遺傳學；候選基因策略；抽穗期；株高；數量性狀基因座；水稻。