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ORIGINAL PAPER

Identification of a novel gene *ef7* conferring an extremely long basic vegetative growth phase in rice

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Abstract A late heading-time mutant line, HS276, which was induced by gamma-irradiation of seeds of the *japonica* rice (*Oryza sativa* L.) variety Gimbozu, exhibits an extremely long basic vegetative growth phase (BVP). A genetic analysis using the F_2 population from the cross between HS276 and Gimbozu revealed that the late heading of HS276 is governed by a single recessive mutant gene. The subsequent analysis on heading responses of HS276 and Gimbozu to four photoperiods (12, 13, 14, and 15 h) and to the photoperiodic transfer treatment from a short photoperiod to a long photoperiod revealed that the mutant gene confers an extremely long BVP and increases photoperiod sensitivity under long photoperiod (14 and 15 h). The BVP durations of HS276 and Gimbozu were estimated at 30.1 and 16.0 days, respectively; the mutant gene, compared with its wild type allele, elongates the duration of BVP by 14 days. Linkage analysis showed that the mutant gene is located in the 129 kb region between the two INDEL markers, INDELAP0399_6 and INDELAP3487_2, on the distal part of the short arm of chromosome 6. None of the other BVP genes are located in this region; therefore, we declared this a newly detected mutant gene and designated it *ef7*. A recently established program to breed rice suitable for low latitudes, where short photoperiodic conditions continue throughout the year, aims to develop varieties with extremely long BVPs and weak photoperiod sensitivities; the mutant gene *ef7*, therefore, will be quite useful in these programs because it confers an extremely long BVP and little enhances photoperiod sensitivity under short photoperiod.

Introduction

Flowering time (also called heading time) plays a principal role in the regional adaptability of plants. Extensive conventional genetic analyses of flowering time have been conducted in the short-day rice plant (*Oryza sativa* L.), and as many as 25 heading-time loci have been identified (Tsai 1986; Poonyarit et al. 1989; Yokoo and Kikuchi 1992; Okumoto and Tanisaka 1997; Ichitani et al. 1998). In addition, many quantitative trait loci (QTLs) for heading time have been reported (Yano et al. 1997; Yamamoto et al. 2000). According to Chang and Vergara (1969), the preflowering development of rice can be divided into three successive phases: the basic vegetative growth phase (BVP); the photoperiod-sensitive phase (PSP), which lasts until panicle initiation; and the reproductive phase (RP), which lasts from panicle initiation to flowering (heading). Their study found that photoperiod length did not influence the duration of BVP or that of RP, and the latter being almost constant at about 35 days, but it did significantly influence the duration of PSP. The authors also indicated that PSP was eliminated under optimum photoperiod conditions. Based on these findings, the heading time of rice is now considered to be chiefly determined by the length of the basic vegetative growth (BVG) period (which is nearly equal to the duration of BVP plus that of RP), expressed in

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days to heading (DH), under optimum conditions of photoperiod and photoperiod sensitivity (PS), which are determined by observing the different numbers of days to heading under various photoperiod conditions (Hosoi 1981; Tanisaka et al. 1992). Yet only six known loci control the duration of BVP: *Ef1* on chromosome 10 (Tsai 1986), *Ef2* on chromosome 9 (Khun et al. 2005b), *Ef3* (chromosome unknown; Tsai 1991), *Ef4* on chromosome 3 (Khun et al. 2005a), *Ef5* on chromosome 6 (Khun et al. 2004), and *Ef6* on chromosome 7 (Khun et al. 2006). *Ehd1* (Doi and Yoshimura 1998) was thought to be another locus controlling the duration of BVP, but we have discovered that *Ehd1* and *Ef1* are in fact the same locus (Saito et al. 2009). Most of the 25 known heading-time loci of rice control PS, and thus affect only the duration of PSP.

A number of genetic studies have been published on flowering time in the long-day plant *Arabidopsis thaliana* (L.) Heynh., which is regarded as a model plant for the study of genome structure of dicots. These have utilized a large number of flowering time and flower-formation mutants induced from various ecotypes and have described the physiological and biochemical functions of many flowering-time genes (Koornneef and Peeters 1997; Reeves and Coupland 2001). In addition, these studies have proposed several genetic pathways to flowering (Hayama and Coupland 2003; Baurle and Dean 2006). These *Arabidopsis* studies provide an example of how useful induced mutations can be in investigations of the genetic mechanism of flowering in plant species. In rice, which is regarded as a model plant for the study of grasses, a few PS loci have been studied, and their physiological and biochemical functions, along with their roles in genetic pathways to flowering, have been gradually disclosed (Yano et al. 2000; Izawa et al. 2002; Lee et al. 2004; Tamaki et al. 2007; Komiya et al. 2008; Matsubara et al. 2008; Park et al. 2008; Wu et al. 2008; Xue et al. 2008). In comparison, little progress has been made in the study of BVP genes, or in the elucidation of their physiology and biochemistry: only the *Ehd1* (=*Ef1*) locus is known to promote heading time, encoding a B-type response regulator (Doi et al. 2004). Genetic studies of the loci controlling the duration of BVP are limited, mainly due to the lack of mutants among these genes.

A recently established rice breeding program serving low latitudes aims to develop rice varieties with long BVPs and weak PSs. Such varieties will thrive under the vegetative growth period conditions found in low latitudes, where short photoperiods persist throughout the year, and will thereby permit stable, high-yielding production in addition to double or triple cropping. Clearly, this program would benefit from the ability to exploit new mutant genes conferring an extremely long BVP; for this purpose, Tanisaka et al. (1992) induced many mutants of the BVP genes. Among these, one late heading-time mutant line called HS276 exhibited an extremely long BVP. In the present study, we attempted to identify the mutant gene(s) conferring the extremely long BVP of HS276, to investigate its heading response to diverse photoperiods, and to estimate the accurate duration of BVP and photoperiod sensitivity. We found that the mutant gene is a nonfunctional allele at the novel locus *Ef7*. Previous estimates of the duration of BVP have required either photoperiodic transfer treatment (Ellis et al. 1992) or microscopic observation of the reproductive shoot apex, but both of these procedures are difficult to perform on a large number of plants. Instead, we conducted genetic analyses of the duration of BVP using the BVG period, viz., the DH under short-photoperiod conditions.

Materials and methods

Plant materials

This study used HS276 (a late heading-time mutant line exhibiting an extremely long BVP), its original variety Gimbozu, the *indica* variety Kasalath, and three chromosome segment substitution lines (CSSLs), SL26, SL28 and SL29. HS276 was induced by means of gamma-irradiation of seeds of the *japonica* rice variety Gimbozu; it headed (flowered) two weeks later than Gimbozu (Tanisaka et al. 1992). The CSSLs all carry a single segment of chromosome 6 from the donor Kasalath-genome and have a pure genetic background from the recurrent parent Nipponbare: SL26 carries a defined segment on the short arm of chromosome 6; SL28 carries a defined segment on the interspatial part of the long arm; and SL29 carries a single defined segment on the distal part of the long arm (cf. Fig. 5; Yano and Ebitani 2002; see also the Rice Genome Resource Center: http://www.rgrc.dna.affrc.go.jp).

Genetic analysis of the mutant gene(s) (Experiment 1)

The F_2 population from the cross between HS276 and its original variety Gimbozu, comprising 291 plants, was subjected to a genetic analysis for heading time under natural photoperiod conditions in 2004. The progeny test was conducted with 35 F_3 lines (18–26 plants/line) and parents (21–26 plants/parent) in 2005. Each F_3 line was the progeny of a randomly selected F_2 plant. Germinated seeds were sown in nursery beds in a green house; 30 days after sowing, the seedlings were transplanted into a paddy field in Kyoto (35°01′ N lat.). Parental lines were planted with their F_2 and F_3 populations. In both years, all seeds were sown on 15 May. N, K_2O, and P_2O_5 fertilizers were applied in quantities of 60, 90, and 90 kg/ha, respectively; plant spacing was 10 × 30 cm. Heading date was recorded for each plant when the first panicle emerged from the leaf sheath of the flag leaf.

Heading response to diverse photoperiods (Experiment 2)

Elsewhere, four groups of HS276 plants were grown under photoperiods of 12, 13, 14, and 15 h, each alongside the original variety Gimbozu. Plants from these four groups were then analyzed for photoperiodic response in natural temperature. Germinated seeds were sown on field soil in a 3.6-L pot and covered with granulated soil. Seedlings were thinned to four plants per pot 14 days after sowing. Five pots for each variety/line were grown under each photoperiod. In addition to natural daylight (8:00 a.m.–6:00 p.m.), supplementary light from incandescent lamps (3.24 Wm^{-2} at the surface of soil) was used to achieve the desired length of photoperiod for all the treatments. Exposure to these photoperiods started on the day of sowing (1 May 2006) and continued for 120 days, and the heading date of the main culm was recorded. All plants that did not head within 120 days after sowing were examined and the developmental stage of the reproductive shoot apex was determined. All tillers except the main culm were cut off whenever they appeared. HYPONeX solution (100 mL) at a 1.3% (w/v) concentration per pot was applied as an additional fertilizer every other week.

Estimation of the duration of BVP (Experiment 3)

Ellis et al. (1992) proposed a model in which they estimated the duration of the developmental phases prior to flowering by a non-linear regression analysis using a data set obtained through photoperiodic transfer treatments in soybean plants. Using the same model, Yin et al. (1997) successfully estimated the duration of the development phase of many *indica* and *japonica* rice varieties, and at the same time demonstrated that actual durations of BVP were generally shorter than estimates based on the method of Chang and Vergara (1969) [the duration of BVP (days) = the BVG period (days) − 35 days)]. We estimated the duration of BVP of HS276 using DH data obtained from the photoperiodic transfer treatment, according to the analytical model of Ellis et al. (1992). The short photoperiod was 10 h, and the long photoperiod was 14 h. Germinated seeds were sown on field soil in a 3.6-L pot and covered with granulated soil. Seedlings were thinned to four plants per pot 14 days after sowing. Five pots for each variety/line were grown under each photoperiod. The photoperiod treatments began on the day of sowing (15 May 2007). The plants were kept in two growth cabinets (Shimazu SCN401, Kyoto, Japan), both of which were kept at 30°C between 6:00 a.m. and 6:00 p.m. and at 26°C between 6:00 p.m. and 6:00 a.m.. Thirteen pots for each variety/line were initially kept under a 14 h photoperiod. Seven days after sowing, one pot per variety/line was transferred to a 10 h photoperiod. Once a pot had been transferred it was grown under a 10 h photoperiod until heading. One pot of HS276 plants was transferred at each of the following intervals: 0, 7, 14, 21, 28, 35, 42, 49, 56, 63, 80, and 90 days after sowing, while one pot of Gimbozu plants was transferred at each of the following intervals: 0, 7, 14, 21, 28, 35, 42, 49, 56, 63, 70, and 77 days after sowing. In addition to 10 h natural daylight (8:00 a.m.–6:00 p.m.), supplementary artificial light from incandescent lamps (3.24 Wm^{-2} at the surface of soil) was used for all photoperiod treatments. Other experimental procedures were similar to those used in Experiment 2.

QTL analysis of the mutant gene (Experiment 4)

The F_2 population from the cross between HS276 and the *indica* variety Kasalath, comprising 96 plants, was grown in a paddy field in Kyoto, and subjected to a QTL (quantitative trait locus) analysis with 64 SSR (simple sequence repeat) markers. The sowing date and the transplanting date were 11 May 2005 and 9 June 2005, respectively. QTLs were identified with simple interval mapping (SIM) and composite interval mapping (CIM), using software packages QTL Cartographer ver. 2.5 (Wang et al. 2005). LOD thresholds for the presence of QTL were estimated by one thousand times permutation test using QTL Cartographer. The LOD-score peak was used to estimate the most likely QTL position on the linkage map. Other experimental procedures were similar to those used in Experiment 1.

Chromosome mapping of the mutant gene(s) (Experiment 5)

Since the mutant gene was already roughly mapped on chromosome 6, we proceeded to the fine mapping of the mutant gene with SSR (simple sequence repeat) markers using three F_2 populations, each ranging from 93 to 192 plants, from the cross between the three CSSLs and HS276. To further narrow down the region of the mutant gene, we used six INDEL markers, which were designed from a publicly available rice genome sequence, as described later. The F_2 populations were grown together with their parental lines under a 10 h photoperiod. The fertilizers and plant spacing applied were similar to those in Experiment 2. Heading date was recorded as in Experiment 2.

Results

Genetic analysis of the mutant gene(s) (Experiment 1)

The F_2 population from the cross between Gimbozu and HS276, comprising 291 plants, showed a bimodal frequency distribution of DH within the parental ranges, with

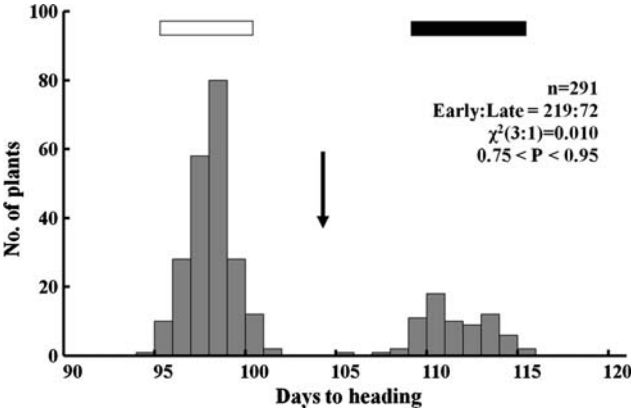

Fig. 1 Frequency distribution of days to heading in the F_2 population from the cross between Gimbozu and HS276. *White* and *black boxes* indicate the ranges of days to heading of Gimbozu and HS276, respectively. *Arrow* indicates the breakpoint which divides the population into early (Gimbozu-type) and late heading (HS276-type) groups

a clear breakpoint dividing the population into early (Gimbozu type) and late (HS276 type) groups (Fig. 1). The ratio of early type (219 plants) : late type (72 plants) fit the 3:1 ratio expected for one-locus segregation ($\chi^2 = 0.01$, $0.75 < P < 0.95$). In the progeny test, all the F_3 lines were easily classified into three groups (Table 1). The ratio of [Gimbozu type]:[segregating type]:[HS276 type] lines was 8:20:7, which fits the 1:2:1 ratio expected for one-locus segregation ($\chi^2 = 0.77$, $0.50 < P < 0.75$). Thus, we determined that the late heading of HS276 is governed by a single recessive mutant gene.

Heading response to diverse photoperiods (Experiment 2)

Heading responses of HS276 and its original variety Gimbozu to photoperiods of 12, 13, 14 and 15 h are shown in Table 2. Under short photoperiods of 12 and 13 h, HS276 headed 8.7 and 10.0 days later than Gimbozu, respectively, which suggested that the mutant gene governing the late heading of HS276 affects the BVP. Under long photoperiods, the delay in heading was more marked in both HS276 and Gimbozu: under a 15 h photoperiod, neither HS276 nor Gimbozu had headed even 120 days after sowing, but examination revealed that the flower primordia of the two varieties had reached different developmental stages: Gimbozu had reached the stage of differentiating pollen mother cells, whereas HS276 had reached the stage of differentiating flower primordia. These results suggest that the mutant gene controls the duration of BVP, and little and considerably increases photoperiod sensitivity under short (at least 13 h) and long (14 and 15 h) photoperiods, respectively.

Estimation of the duration of BVP (Experiment 3)

Heading responses of HS276 and Gimbozu plants that were subjected to the transfer treatment from long photoperiod to short photoperiod corresponded well with the model of Ellis et al. (1992) (Fig. 2). The DHs of both HS276 and Gimbozu were constant in plants that had been transferred early, but gradually increased with the length of time that passed before the transfer. Using the analytical model, we successfully estimated the duration of BVP and the degree

Table 1 Frequency distributions of days to heading in representative F_3 lines (Gimbozu/HS276)

Cross parents and F_2 plants (genotype)	Days to heading																							Total number of plant
	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125	126	
Gimbozu				11	7	3																		21
HS276														2		2	11	4	2	3	2			26
Gimbozu homozygous			1	5	6	6	5		1															24
				9	4	11	2																	26
				2	2	4	17	1																26
				3	3	8	6			1														21
Heterozygous				7	3	2	3	1						2	2	2	4							26
				4	7	2	2	1	1							1		3						21
				2	3	2	4		1						3	4	2		1					22
				1	1	3	6	3	2									1	2		2			21
					3	3	7	1	1	1				2	2	1								21
					1	4	8	3	1	1					2	1	4							25
HS276 homozygous														2	4	10	3	1						20
														1	3	2	1	3	5	2	1			18
															2	2	2	9	2	1	1			19
															1	6	4	3	4	1	1			20

Table 2 Heading responses to diverse photoperiods (12, 13, 14 and 15 h) of the late heading mutant line HS276 and its original variety Gimbozu

Line	Days to heading ± SE			
	12 h	13 h	14 h	15 h
HS276	55.1 ± 1.26	63.3 ± 1.05	100.3 ± 3.23	>120
Gimbozu	46.4 ± 0.55	53.3 ± 0.70	80.4 ± 3.56	>120

of PS expressed by regression coefficient (*b*) of DH on transfer time during PSP under a short photoperiod (Fig. 2). The BVP duration of Gimbozu was 16.0 days, while that of HS276 was 30.1 days. Thus the mutant gene was estimated to have increased the duration of BVP by 14.1 days. HS276 exhibited a slightly larger *b* value (0.6) than Gimbozu (0.5), although there was no significant difference between the values (Fig. 2). Since *b* is an index of photoperiod sensitivity (Ellis et al. 1992), this implies that the mutant gene does not greatly modify the duration of PSP under short photoperiods.

QTL analysis of the mutant gene(s) (Experiment 4)

Restriction fragment length polymorphism (RFLP) and simple sequence repeat polymorphism (SSR) markers have been used in many species to identify the locations of various kinds of genes. Actually, these two markers appear in large numbers between *indica* and *japonica* rices (McCouch et al. 1988; Kurata et al. 1994). But RFLP and SSR are rarely visible between genetically close *japonica* rices (Akagi et al. 1997). Therefore, we conducted a QTL analysis using the F_2 population of the cross between HS276 and the *indica* variety Kasalath under a natural photoperiod. A total of seven QTLs for days to heading were detected with SIM and CIM analyses (Fig. 3). Yano et al. (1997) detected five QTLs, *Hd3a*, *Hd1*, *Hd2*, *Hd4* and *Hd5*, in the F_2 population of the Nipponbare/Kasalath cross. According to Monden et al. (2009), one major QTL on the short arm of chromosome 6 was contributory to the heading time difference between Gimbozu and Nipponbare, indicating that those two varieties have the same genotypes for heading time except this major QTL. Accordingly, in the F_2 population of the HS276/Kasalath cross, two QTLs, including the mutant locus, in addition to the five (*Hd3a*, *Hd1*, *Hd2*, *Hd4*, and *Hd5*) would be detected. Actually, out of the seven QTLs, two on chromosome 6, two on chromosome 7, and one on chromosome 8 showed almost the same chromosomal positions as those of *Hd3a*, *Hd1*, *Hd2*, *Hd4*, and *Hd5*, respectively. Therefore, these five QTLs could be excluded from the candidate of the mutant gene. Thus we considered that either of the remaining two QTLs on chromosome 6 was the candidate of the mutant locus.

Chromosome mapping of the mutant gene(s) (Experiment 5)

To obtain a clear-cut segregation of the mutant gene locus, we crossed HS276 with the three CSSLs, SL26, SL28 and SL29, each of which carried a single defined segment of chromosome 6 of the Kasalath genome in the genetic background of *japonica* variety Nipponbare. A linkage analysis was conducted using the F_2 populations grown under a 10 h photoperiod. The F_2 population from the SL28/HS276 cross showed a continuous distribution with many transgressive segregants (Fig. 4c). It is therefore considered that this cross combination was not relevant for the linkage analysis of the mutant gene. In contrast, the F_2 populations of the SL26/HS276 and SL29/HS276 crosses each showed a bimodal distribution with a clear breakpoint, which divided each population into early (SL26 or SL29 type):

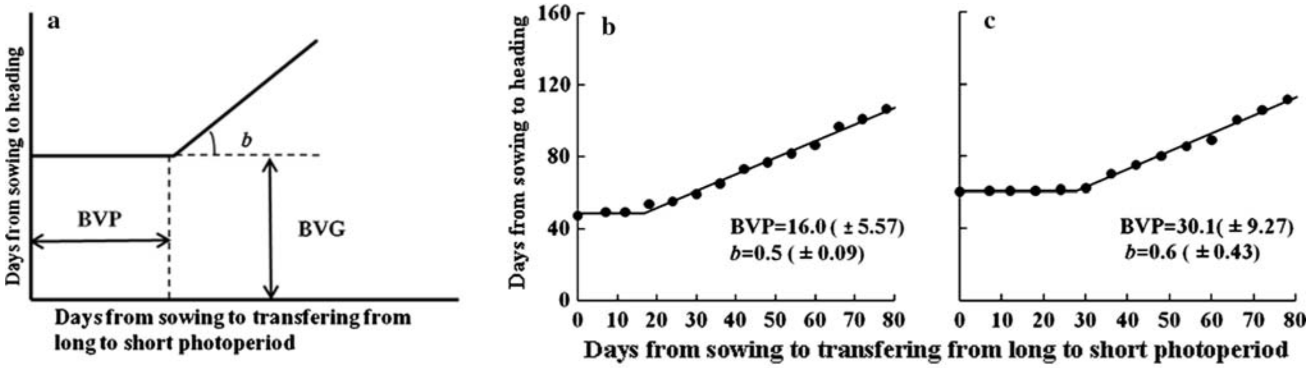

Fig. 2 Heading responses of Gimbozu and HS276 to the photoperiodic transfer treatment from long photoperiod (LP) to short photoperiod (SP). **a** Schematic representation of heading responses of plants transferred from LP to SP at various times after sowing. *Arrows* indicate the duration of the basic vegetative growth phase (BVP) and the basic vegetative growth (BVG) period. *b* indicates regression coefficient of days to heading during the photoperiod sensitive phase on days from sowing to transfer treatment from LP to SP. **b** Heading response to transfer treatment of Gimbozu. **c** Heading responses to transfer treatment of HS276

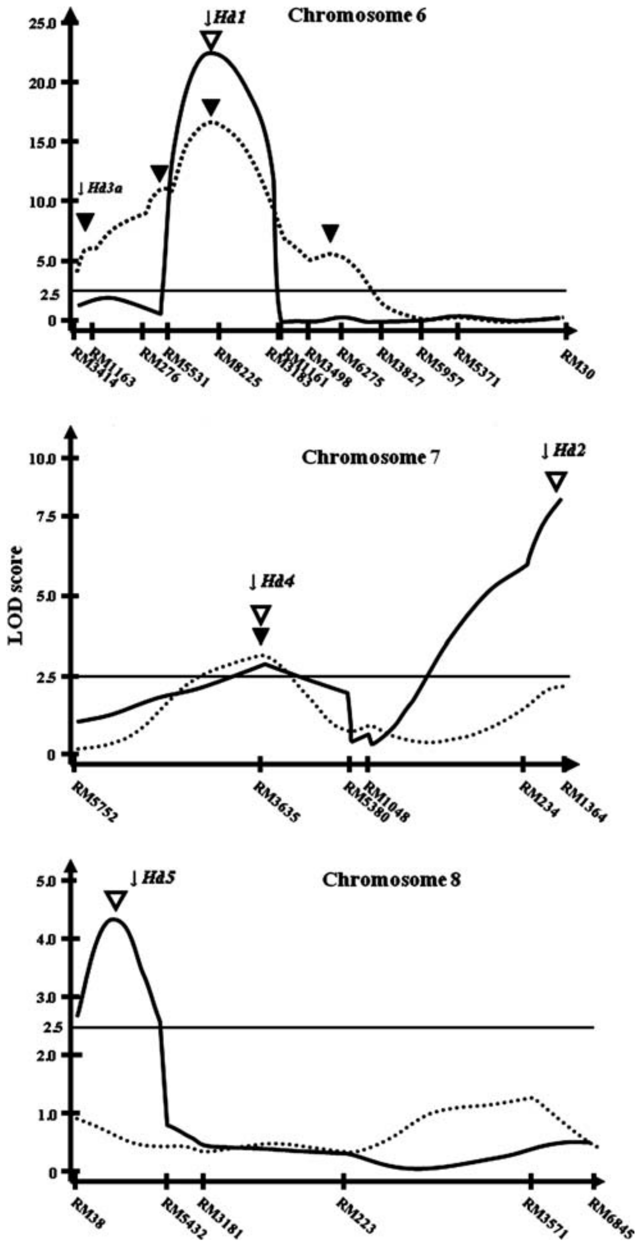

Fig. 3 QTL LOD score plots for heading time of 96 plants derived from the cross combination between HS276 and Kasalath. *Black* and *broken lines* indicate the results from composite interval mapping (CIM) method and single interval mapping (SIM) analyses, respectively. *Open* and *black triangles* indicate the peak positions of the candidate QTLs from CIM and SIM method, respectively. *Arrows* indicate the QTLs detected in the F_2 population of the Nipponbare/Kasalath cross (Yano et al. 1997)

late (HS276 type). In both of these cross combinations, the segregation ratio fit the 3:1 ratio expected for one-locus segregation ($\chi^2 = 0.448$: $0.50 < P < 0.75$ and $\chi = 0.172$: $0.75 < P < 0.95$, respectively) (Fig. 4a, d).

Next we investigated the linkage relationships between the mutant gene and SSR markers using the two F_2 populations. We observed no linkage relationships between the mutant gene *ef7* and DNA markers located within the region between RM3827 and the end of long arm of chromosome 6 in the F_2 population of the SL29/HS276 cross. It was therefore considered that the mutant gene was not located on the long arm of chromosome 6. To the contrary, in the F_2 population of the SL26/HS276 cross, we found that the mutant gene *ef7* was closely linked to a SSR marker RM3414 with a physical distance of 0.54 cM (Fig. 4b). Using 1857 F_2 plants of the SL26/HS276 cross, we attempted to narrow down the region of the mutant gene by using INDEL markers which we designed from a publicly available rice genome sequence (RAP-DB: http://rapdb.dna.affrc.gp.jp/). In developing these INDEL markers, we predicted the likelihood of detecting any polymorphism between HS276 and Kasalath by comparing the sequence of the *japonica* variety Nipponbare (sequenced by RAP-DB) with that of the *indica* variety 93–11 (sequenced by the Beijing Genomics Institute; http://rice.genomics.org.cn/index.jsp). Primer sequences of the five newly developed INDEL markers used in this study are listed in Table 3. Through our experiments with these markers, the chromosomal location of the mutant gene was narrowed down to a region with a physical length of less than 129 kb, the region between the two INDEL markers, INDELAP0399_6 and INDELAP3487_2, which is on the short arm of chromosome 6 (Fig. 5; Table 4).

Discussion

The objective of the present study was to investigate the genetic factor(s) conferring the extremely long BVP of HS276, and to investigate that factor's physiological effect on the pre-flowering developmental phase. We successfully identified a single recessive mutant gene conferring an extremely long BVP and slightly increases photoperiod sensitivity. This mutant gene proved to be located in the 129 kb region between two INDEL markers, INDELAP0399_6 and INDELAP3487_2, on the distal part of the short arm of chromosome 6.

Seven loci involved in the BVP have been identified prior to this publication. Among them, only *ef5* is located on the short arm of chromosome 6. According to Khun et al. (2004), *ef5* is linked to the *wx* (*glutinous endosperm*) gene with a recombination value of 12.9%. Its position is on the long arm side of the *wx* gene. In contrast, the mutant gene controlling the BVP of HS276, although closely linked to *wx*, is located on the short arm side. Accordingly, it is clear that the mutant gene is non-allelic to the *ef5* gene.

The estimated chromosome region of the mutant gene includes the RFLP marker R1952, which is one of the flanking markers of *Hd3b* (*Heading date-3b*) (Monna et al. 2002). In addition to *Hd3b*, this region also contains two

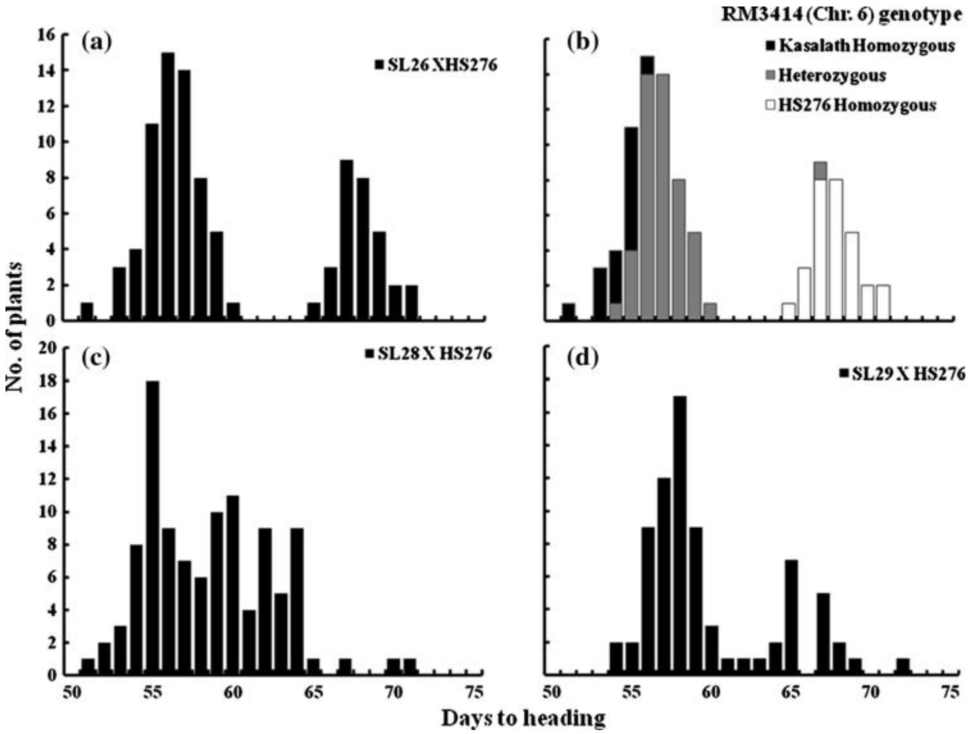

Fig. 4 Frequency distributions of days to heading in the F_2 populations from the crosses of SL26 × HS276 (**a**, **b**), SL28 × HS276 (**c**), and SL29 × HS276 (**d**)

Table 3 List of four SSR and five INDEL markers and their primer sequences used in this study

Marker	Forward primer	Reverse primer
RM6003	GGGTTGGTCACTCACAGCTTGC	TTCCATCACGTCTCTCGTCTTCC
RM587	TTCCCATCTGCACTACCATAATCC	GAGCAGAGATGTGCTTTGCTACC
RM19363	GGGATTAGGGCGACGAGAAGAGG	ACGGCCACGACTCCATCTCTCC
RM19381	AACGGGAGATCACAGGAATTTGC	GTGTTCGACTCGTCTCCATTTCG
INDELAP0399_1	TGACCATACCGCACAGAGAC	AATCAAATCAAGCCCCCTTC
INDELAP0399_4	GGGCTAGATTGGTGCCTTTG	GCAGACAAGAGGGTGGTAGC
INDELAP0399_6	GCGTTGTGTTGTGATTTTGC	AATATGCTGGTGGCCATACAC
INDELAP0399_7	TGAGGAAGCTGACAATGAGC	GGCCCGGAAAAATTATACTCC
INDELAP3487_2	ATAATGAAGCCAGGGCGAAG	CATCGTTGGAGATGGATGG

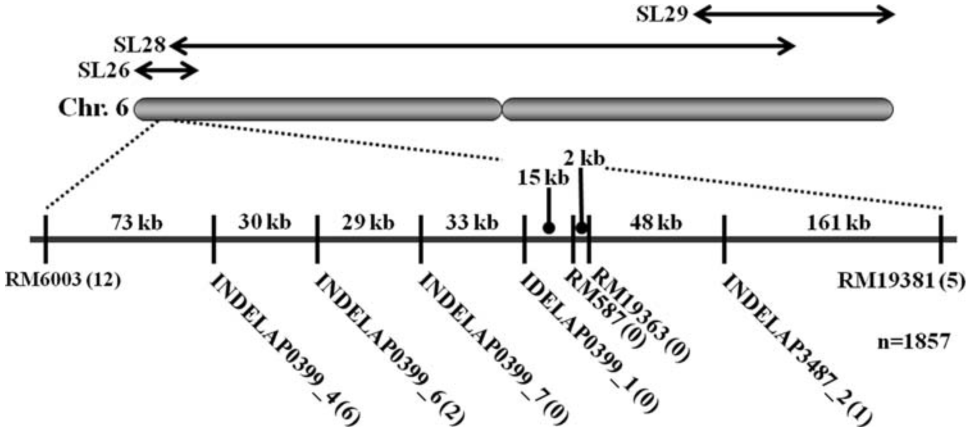

Fig. 5 Location of the mutant gene of HS276 and marker positions on chromosome 6. Number in parenthesis indicates number of recombinant plants. *Arrows* on the *top* indicate the region substituted by Kasalath-chromosome in each CSSL line

heading-time-related genes, *Hd3a* (*Heading date-3a*) and *RFT1* (*Rice flowering time-1*). We had to assess whether the mutant gene controlling the BVP of HS276 was allelic to any of these genes. A functional allele at the *Hd3a* locus does not affect heading time under long photoperiods, but it does promote heading under short photoperiods. This

Table 4 Days to heading and graphical genotypes of representative F_2 recombinants between RM6003 and RM19381 in the F_2 population of the HS276/SL26 cross

F_2 individual no.	Marker genotype[a]									Days to heading	*Ef7* genotype[b]
	RM6003	INDEL AP0399_4	INDEL AP0399_6	INDEL AP0399_7	INDEL AP0399_1	RM19363	RM587	INDEL AP3487_2	RM19381		
11-2-26	HS	HS	HS	HS	HS	HS	HS	HS	HS	64	HS
24-1-30	HS	HS	HS	HS	HS	HS	HS	HS	H	69	HS
14-1-45	H	HS	HS	HS	HS	HS	HS	HS	HS	67	HS
16-1-32	H	H	HS	HS	HS	HS	HS	HS	HS	67	HS
17-2-39	H	H	H	HS	HS	HS	HS	HS	HS	66	HS
17-2-10	H	H	H	H	H	H	H	HS	HS	58	SL
18-2-28	H	H	H	H	H	H	H	H	HS	58	SL
18-1-22	HS	HS	HS	H	H	H	H	H	H	59	SL
23-2-41	HS	HS	H	H	H	H	H	H	H	56	SL
26-2-14	HS	H	H	H	H	H	H	H	H	57	SL
11-3-40	H	H	H	H	H	H	H	H	H	57	SL
24-2-3	K	K	K	K	K	K	K	K	K	54	SL

[a] *HS* HS276-type homozygote, *H* heterozygote, *K* Kasalath-type homozygote

[b] *HS* HS276 (late heading) type, *SL* SL26 (early heading) type

suggests that the *Hd3a* locus controls the duration of BVP. Yet no sequence polymorphism was present in the ORF region of *Hd3a* among Nipponbare, Gimbozu and HS276, suggesting that the mutant gene is non-allelic to *Hd3a*. As for *RFT1*, no sequence difference was found between Gimbozu and HS276, although there was a slight sequence difference between Nipponbare and Gimbozu. Thus the mutant gene is non-allelic to *RFT1*. We could not compare the *Hd3b* sequence of HS276 with those of Gimbozu and Nipponbare, since *Hd3b* has not yet been sequenced. According to Monna et al. (2002), *Hd3b* did not have a strong effect on heading time under short photoperiods, but it delayed heading to a remarkable degree under long photoperiods. This suggests that *Hd3b* is involved in photoperiod sensitivity and has little effect on the duration of BVP. It is therefore considered that the mutant gene, which elongates the duration of BVP considerably, is non-allelic to *Hd3b*. Thus the mutant gene was considered to be non-allelic to all known heading-time genes. We designated this newly detected mutant gene of HS276 *ef7* (its wild type allele is *Ef7*).

Experiment 2, which was conducted under natural temperature conditions, showed that *ef7* increases photoperiod sensitivity. We also evaluated the effects of *ef7* on the photoperiod sensitivity with the photoperiodic transfer treatment in the growth cabinet where temperature was artificially controlled (Experiment 3); consequently, we found that the regression coefficient (b) of days to heading on transfer time, which indicates the strength of photoperiod sensitivity (Yin et al. 1997; Nishida et al. 2001), was not significantly different between Gimbozu ($b = 0.5$) and HS276 ($b = 0.6$). Yin et al. (1997) showed that PSP was not eliminated even under an optimum photoperiod, and termed such a phase PSP_S (photoperiod sensitivity under short photoperiod) and another photoperiod-sensitive phase that delays heading in response to LP PSP_L (photoperiod sensitivity under long photoperiod). In Experiment 2, the delayed heading of HS276 was observed under long photoperiods. It is therefore considered that the difference in photoperiod sensitivity detected in Experiment 2 might be attributable to the difference in the duration of PSP_L.

Using the photoperiodic transfer treatment, we were able to estimate the accurate durations of BVP, not including the RP period, of both HS276 (30.1 days) and Gimbozu (16.1 days). Gimbozu and HS276 have the same genotypes for all heading-time loci other than *Ef7* (Fig. 1); therefore, *ef7* can be assumed to be responsible for the entire increase in BVP duration, which is estimated to be ca. 14 days. Nishida et al. (2001) likewise used the photoperiodic transfer treatment to estimate the increase in BVP duration caused by the recessive allele *ef1* at 15.9 days, an increase which is almost equal to that caused by *ef7*. In a later publication (Nishida et al. 2002), however, the same research group stated that *ef1* requires the interaction of *Se1-e* at the *Se1* in order to realize an extremely long BVP, indicating that *ef1* permits some residual function to accelerate heading by shortening the BVP duration. The present study did not establish whether *ef7* also permits some residual function; further analysis will be needed to clarify that point.

According to the Chang and Vergara (1969) method of estimating BVP duration, the BVP durations of HS276 and Gimbozu were estimated at 20.1 and 11.4 days, respectively.

These estimated values are noticeably different from the actual values we observed in the present study. This discrepancy indicates that the duration of RP is not constant at 35 days. An accurate estimate of BVP duration will require either the photoperiodic transfer treatment (Ellis et al. 1992) or microscopic observation of the reproductive shoot apex.

The double recessive mutant line for the *Ef1* and *Ef7* loci exhibited a far longer BVP duration (data not shown) than the two single recessive mutant lines, both of which were induced from the strongly photoperiod-sensitive variety Gimbozu. Thus *ef1* and *ef7* seem to regulate the duration of BVP independently. It is known that one function of the *Ef1* locus is to induce the expressions of two florigen-like genes, *Hd3a* and *RFT1* (Doi et al. 2004). The present study could not determine how these two genes were expressed in HS276; a future study that answers this question will constitute a significant contribution to the study of genetic pathways to flowering in rice.

The identification of the genes elongating the duration of BVP will play an important role in the recently established rice breeding program for low latitudes, which aims to develop varieties with long BVPs and weak photoperiod sensitivities. The mutant gene *ef7*, identified in the present study, confers an extremely long BVP (BVG period), and therefore it will be particularly useful in the low latitude rice breeding program.

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