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ORIGINAL PAPER

Accumulation of additive effects generates a strong photoperiod sensitivity in the extremely late-heading rice cultivar 'Nona Bokra'

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Abstract Many rice cultivars that originated from lower-latitude regions exhibit a strong photoperiod sensitivity (PS) and show extremely late heading under long-day conditions. Under natural day-length conditions during the cropping season in Japan, the *indica* rice cultivar 'Nona Bokra' from India showed extremely late heading (202 days to heading) compared to the *japonica* cultivar 'Koshihikari' (105 days), from Japan. To elucidate the genetic factors associated with such extremely late heading, we performed quantitative trait locus (QTL) analyses of heading date using an F_2 population and seven advanced backcross progeny (one BC_1F_2 and six BC_2F_2) derived from a cross between 'Nona Bokra' and 'Koshihikari'. The analyses revealed 12 QTLs on seven chromosomes. The 'Nona Bokra' alleles of all QTLs contributed to an increase in heading date. Digenic interactions were rarely observed between QTLs. Based on the genetic parameters of the QTLs, such as additive effects and percentage of phenotypic variance explained, these 12 QTLs are likely generate a large proportion of the phenotypic variation observed in the heading dates between 'Nona Bokra' and 'Koshihikari'. Comparison of chromosomal locations between heading date QTLs detected in this study and QTLs previously identified in 'Nipponbare' × 'Kasalath' populations revealed that eight of the heading date QTLs were recognized nearby the *Hd1*, *Hd2*, *Hd3a*, *Hd4*, *Hd5*, *Hd6*, *Hd9*, and *Hd13*. These results suggest that the strong PS in 'Nona Bokra' was generated mainly by the accumulation of additive effects of particular alleles at previously identified QTLs.

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Introduction

Heading date in rice is a complex trait that is governed by multiple genes and environmental factors, such as day-length, temperature, and soil conditions. During the last decade, genetic studies using DNA markers have facilitated the genetic dissection of heading date (reviewed by Yano et al. 2001), and many quantitative trait loci (QTLs) for heading date have been identified using several mapping populations (Lu et al. 1996; Xiao et al. 1996; Yano et al. 1997; Lin et al. 1998; Septiningsih et al. 2003; Thomson et al. 2003; Fujino and Sekiguchi 2005a, b). In particular, 15 QTLs for heading date (*Hd1–Hd3a*, *Hd3b–Hd14*) have been identified in several populations derived from crosses between 'Nipponbare', a rice cultivar from Japan, and 'Kasalath', a rice cultivar from India (Yano et al. 1997; Lin et al. 1998, 2002, 2003a; Yamamoto et al. 2000; Monna et al. 2002; reviewed by Yano et al. 2001). Nine of these

QTLs (*Hd1*, *Hd2*, *Hd3a*, *Hd3b*, *Hd4*, *Hd5*, *Hd6*, *Hd8*, and *Hd9*) have been mapped as single Mendelian factors (Yamamoto et al. 1998, 2000; Lin et al. 2002, 2003a; Monna et al. 2002; Takeuchi et al. 2003), and studies have shown that *Hd1*, *Hd2*, *Hd3a*, *Hd3b*, *Hd5*, and *Hd6* are involved in day-length response (Yamamoto et al. 2000; Lin et al. 2000, 2003a; Monna et al. 2002). Recently, four QTLs for heading date (*Hd1*, *Hd6*, *Hd3a*, and *Ehd1*) were isolated using a map-based strategy (Yano et al. 2000; Takahashi et al. 2001; Kojima et al. 2002; Doi et al. 2004). These studies have markedly improved our understanding of the genetic control mechanisms underlying photoperiodic response of flowering in rice and have revealed both conserved and divergent features of genetic control of flowering between rice, a short-day plant, and *Arabidopsis*, a long-day plant (Yano et al. 2001; Hayama et al. 2003; Izawa et al. 2003; Hayama and Coupland 2004).

Heading date is an agronomically important trait that determines the regional and seasonal adaptation of rice cultivars (Oka 1958). In particular, photoperiod sensitivity (PS) was shown to be the most important trait in determining the heading date (Hosoi 1976). Oka (1958) suggested that photoperiodic adaptation may have occurred with the distribution of rice cultivars to different regions, and rice cultivars have been classified into sensitive or insensitive groups according to their response to photoperiod. Only photoperiod-insensitive cultivars are found in northern temperate regions, such as Hokkaido in Japan, and in equatorial regions, whereas both sensitive and insensitive cultivars exist in regions between these two extremes. For example, among the photoperiod-sensitive cultivars, those that originated from lower latitudes tend to show greater PS than those from higher latitudes. Thus, a geographical cline exists with regard to PS among rice cultivars, and a wide range of natural variation in heading date has been observed among the cultivars across this geographical cline (Oka 1958). Although the studies cited above provided a better understanding of the genetic control of heading date in rice, the genetic mechanisms underlying this wide range of variation remain to be clarified. For example, the genetic factors responsible for extremely early or late heading still need to be analyzed at the genetic and molecular levels.

In the breeding of rice in Japan, only a few *japonica* cultivars have been used as parental material. To introduce useful agronomic characters such as disease resistance and stress tolerance, however, breeding programs need to hybridize these *japonica* cultivars with exotic genetic resources, such as tropical landraces that originated at lower latitudes. Most modern rice cultivars grown in Japan exhibit relatively early heading and weak or no PS. Because Japan is located at high latitudes and its cropping season has a long day-length compared with tropical regions, many tropical rice landraces show extremely late heading or no heading and strong PS when grown under natural day-length conditions in Japan.

Therefore, for efficient breeding between early and extremely late-heading cultivars, it is important to elucidate the genetic factors involved in extremely late heading in rice. Yokoo et al. (1980) performed genetic analyses in several cultivars with heading dates ranging from extremely early to extremely late. They reported that some late-heading *japonica* cultivars carried a late allele of the locus *Lm* (= *Se1* and *Hd1*) and other late-heading genes, although the genetic mechanism of extremely late heading remained unclear. Other studies used genetic material derived from early- or intermediate-heading cultivars to identify QTLs associated with heading date in rice (Lu et al. 1996; Xiao et al. 1996; Yano et al. 1997).

The *indica* cultivar 'Nona Bokra', which originated in India, requires more than 200 days from sowing to heading (days to heading, DTH) under the natural day-length conditions of Tsukuba, Japan (36°N). Thus, 'Nona Bokra' appears to be an extremely late-heading cultivar with a strong photoperiodic response and would be a good model for studies of these genetic mechanisms. To elucidate the genetic factors conferring an extremely late heading date, we performed a comprehensive genetic dissection of heading date using an F_2 population and advanced backcross progeny derived from a cross between 'Nona Bokra' and the *japonica* cultivar 'Koshihikari'. Twelve QTLs were detected on seven chromosomes, and their chromosomal locations were coincident with those of previously identified QTLs for PS. Therefore, it appears that extremely late heading is generated mostly by the combination of alleles at the detected QTLs.

Materials and methods

Plant materials

The rice cultivars 'Nona Bokra' and 'Koshihikari' were used to produce mapping populations. 'Nona Bokra' is an *indica* cultivar showing extremely late heading in Tsukuba, Ibaraki, Japan, and 'Koshihikari' is a Japanese elite *japonica* cultivar showing relatively early heading. An F_2 population derived from a cross between 'Nona Bokra' and 'Koshihikari' was developed by Lin et al. (2003b). To detect QTLs for heading date, we backcrossed an F_1 plant of 'Nona Bokra' × 'Koshihikari' with 'Koshihikari' as a recurrent parent. We then performed additional backcrossing with 'Koshihikari' to produce more mapping populations from these generations for further genetic analysis. Among the 34 BC_1F_1 plants, we selected one plant in which at least the chromosome regions of QTLs detected in the F_2 population were homozygous for 'Koshihikari' alleles,

based on a whole-genome survey using 98 RFLP markers. Among the BC_2F_1 plants, we selected six in which one or two chromosomes were heterozygous but almost all other regions were homozygous for 'Koshihikari' alleles, based on a whole-genome survey using 82 RFLP markers. Self-pollinated progeny of these populations were used to detect QTLs for heading date.

Evaluation of day-length response

'Nona Bokra' and 'Koshihikari' were grown under three different environmental conditions: natural field conditions in Tsukuba from April to November (NF) or long-day (LD; 14.5 h light) or short-day conditions (SD; 10 h light) in a controlled growth cabinet (Especmic TGEH-9, Tokyo, Japan). This trial was conducted using a completely randomized design. DTH was scored in ten plants per cultivar, and mean values were calculated for each cultivar.

Scoring of DTH in mapping populations

The parental lines 'Nona Bokra' and 'Koshihikari' and 147 F_2 plants were grown in a paddy field at the National Institute of Agrobiological Sciences (NIAS) in Tsukuba, Japan, from April to September 2000. We monitored DTH of each plant as the appearance of the first panicle based on the criteria of Yano et al. (1997). F_2 plants that did not head by the middle of September and 'Nona Bokra' plants were transferred from the paddy field to a greenhouse to monitor DTH in extremely late-heading plants. In addition, the one BC_1F_2 population ($n = 90$) and six BC_2F_2 populations (BC_2F_2-1, -2, -3, -4, -5, and -6: $n = 99$, 88, 100, 91, 100, and 83, respectively) were raised in the paddy field at NIAS from April to September in 2002 and 2003, respectively. DTH was scored in each plant using the same criteria as used for the F_2 population.

Construction of linkage maps and QTL analyses

QTL analysis of the F_2 population was performed using genotype information based on RFLP markers and a linkage map previously constructed by Lin et al. (2003b). A total of 145 RFLP markers distributed on the 12 rice chromosomes were used for the QTL analysis. These markers included those RFLP markers nearest to 15 QTLs (*Hd1*–*Hd3a*, *Hd3b*–*Hd14*) previously reported in populations derived from crosses between 'Nipponbare' and 'Kasalath' (Fig. 1). If the nearest markers did not show polymorphism between 'Nona Bokra' and 'Koshihikari', another neighboring marker was used instead. For QTL analyses of advanced backcross progeny, we genotyped RFLP and STS markers located in the heterozygous chromosomal regions in each population (40, 27, 21, 22, and 32 markers were mapped in the BC_1F_2 and BC_2F_2-3, -4, -5, -6 populations, respectively). In general, we used the RFLP markers mapped in the F_2 population as common markers to identify QTLs in advanced backcross progeny. In these analyses, however, we used the RFLP marker C235 and STS marker E1178SA instead of C764 and R1679, respectively, because they were more closely linked to *Hd1* and *Hd3a*. Total DNA of plants was extracted from leaves by the CTAB method (Murray and Thompson 1980). RFLP and STS analysis were carried out following the procedure described by Harushima et al. (1998) and Wu et al. (2002), respectively. Linkage maps were constructed using MAPMAKER/EXP 3.0 (Lander et al. 1987) based on the genotype data obtained.

Putative QTLs were detected by the composite interval mapping (CIM) function of QTL Cartographer 2.0 (Basten et al. 1994). The threshold for CIM was based on results of 1,000 permutation tests using a 5% level of significance (Churchill and Doerge 1994). The additive and dominant effects and phenotypic variance explained by each QTL were estimated at the marker nearest to the peak LOD score. The total phenotypic variance explained by all the detected QTLs was estimated by the multiple interval mapping (MIM) model of QTL Cartographer 2.0 (Basten et al. 1994). To detect epistatic interactions between the detected QTLs, we performed two-way ANOVA using genotype data of the nearest marker to each QTL.

Results

Difference in PS between the two parental lines

DTH of 'Nona Bokra' and 'Koshihikari' were investigated under NF conditions and two controlled day-length conditions, SD and LD. 'Nona Bokra' showed extremely late heading compared to 'Koshihikari' under the NF and LD conditions (Table 1). In the NF treatment, the average DTH in 'Koshihikari' ($n = 10$) was 105 days and that in 'Nona Bokra' ($n = 10$) was 202 days. In contrast, in the SD treatment, 'Nona Bokra' (59.7 days) and 'Koshihikari' (56.4 days) exhibited approximately the same DTH. These results suggested that 'Nona Bokra' showed a markedly stronger response to day-length than that of 'Koshihikari'.

QTL analysis of F_2 population

DTH in the F_2 population showed a normal distribution ranging from 132 to 193 days, within the range of the parental values, and transgressive segregation was not observed (Fig. 2). Four QTLs for DTH were detected on chromosomes 3, 6, and 7 (Table 2; Fig. 1). The total amount of phenotypic variance explained by all the

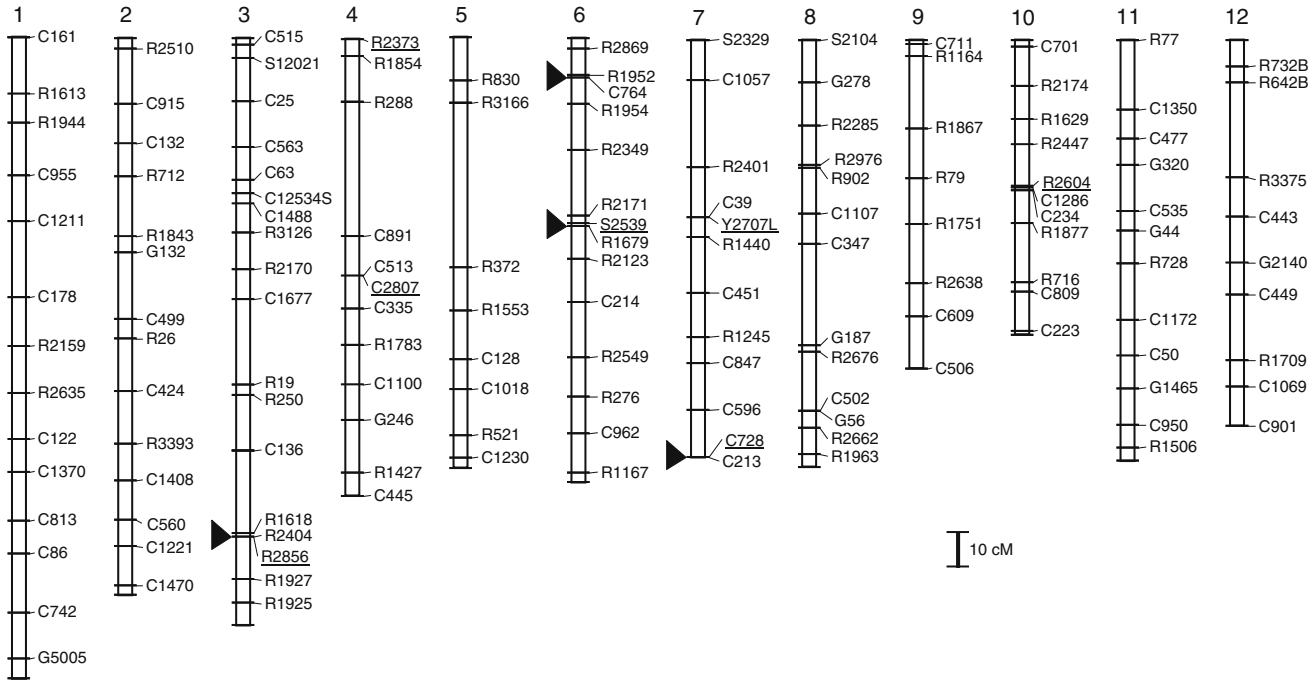

Fig. 1 The positions of QTLs for days to heading in the F_2 population. Chromosome numbers are indicated above each linkage map. The marker loci used are oriented on the framework of the linkage map of 'Nipponbare' × 'Kasalath' constructed by Harushima et al. (1998). Marker names are *indicated on the right* of each chromosome. Underlined markers were not mapped in this population but are shown to indicate the positions of nearest markers to *Hd1* to *Hd14*. Nearest markers to *Hd1* to *Hd14* reported previously are as follows; S2539 (*Hd1*), C728 (*Hd2*), C764 (*Hd3a*), Y2707L (*Hd4*), R1952 (*Hd3b*), R2976 (*Hd5*), R2856 (*Hd6*), C560 (*Hd7*), C12534S (*Hd8*), S12021 (*Hd9*), C2807 (*Hd10*), R2373 (*Hd11*), G56 (*Hd12*), R3375 (*Hd13*), and R2604 (*Hd14*). *Arrowheads* indicate the location of markers nearest to the QTLs detected

Table 1 Differences in days to heading between the 'Nona Bokra' and 'Koshihikari' under three different environmental conditions

Parental variety	Days to heading		
	Short-day condition (mean ± SD)	Long-day condition (mean ± SD)	Natural field condition (mean ± SD)
'Nona Bokra'	59.7 ± 2.9[a]	NA	202.0 ± 2.0[b]
'Koshihikari'	56.4 ± 1.3[a]	75.7 ± 1.9[c]	105.2 ± 1.1[d]

Means within a column followed by different letters are significantly different at $P < 0.01$

NA indicates that 'Nona Bokra' was grown for more than 160 days in the growth cabinet but showed no heading; at this stage, we did not observe any transition of meristem from vegetative to reproductive growth

detected QTLs was 66.5% based on the MIM analysis. The phenotypic variance explained by each QTL (R^2) ranged from 5.5 to 25.1%. The additive effects of the 'Nona Bokra' alleles of the four QTLs ranged from 4.6 to 9.0 days, respectively (Table 2). Two-way ANOVA revealed a weak epistatic interaction between a QTL near R2404 and one near R1679 ($P = 0.050$). A comparison of the chromosomal locations of the four QTLs detected in this study and 15 QTLs previously identified in populations derived from crosses between 'Nipponbare' and 'Kasalath' (Yano et al. 2001) revealed that the four QTLs were near *Hd6*, *Hd3a*, *Hd1*, and *Hd2*, respectively. Based on the genetic parameters of the QTLs, most of phenotypic variation for DTH in the F_2 population seemed to be explained by these four QTLs. However, the difference in DTH between the parental cultivars 'Nona Bokra' and 'Koshihikari' could not be fully explained by these QTLs, suggesting that some additional QTL(s) for DTH remained to be found. Thus, we analyzed the advanced backcross progeny.

QTL analyses of advanced backcross progeny

The average DTH in 2002 for 'Koshihikari' ($n = 10$) was 103 days, whereas no heading was observed in 'Nona Bokra' during the cropping season. DTH in the BC_1F_2 population ranged from 105 to 124 days. Two QTLs for DTH were detected on chromosome 3 (Table 2; Fig. 3). The R^2 was 38.4% at C515 and 11.7% at C1677. The additive effects of the 'Nona Bokra' alleles of the QTLs were 4.1 days at C515 and 2.9 days at C1677. The QTL in the vicinity of C515 was located near *Hd9*, which was identified previously in the 'Nipponbare' × 'Kasalath' populations (Yano et al. 2001). The QTL near C1677 did not match any of the 15 QTLs detected previously in the 'Nipponbare' × 'Kasalath' populations, but it was located

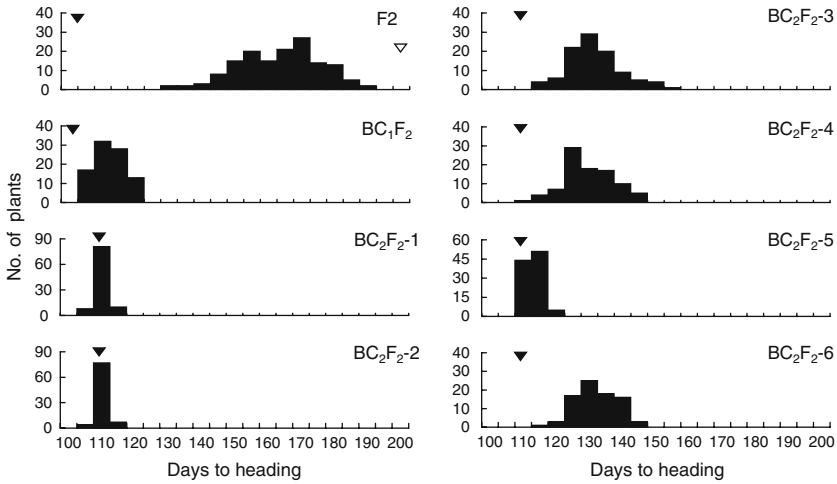

Fig. 2 Frequency distributions of days to heading in the F_2 and seven advanced backcross progeny derived from 'Nona Bokra' × 'Koshihikari'. *White* and *black arrowheads* indicate the average number of days to heading in 'Nona Bokra' and 'Koshihikari', respectively, under natural field condition in Tsukuba, Japan

Table 2 Putative QTLs for days to heading detected in the F_2 and five advanced backcross progeny derived from 'Nona Bokra' × 'Koshihikari'

Population	Chromosome	Nearest marker	LOD[a]	A[b]	SE[c]	D[d]	SE[e]	R^2 [f]	Candidate QTL[g]
F_2	3	R2404	17.4	8.1	±0.061	−3.4	±0.084	21.1	*Hd6*
	6	C764	10.6	5.7	±0.057	4.3	±0.076	11.1	*Hd3a*
	6	R1679	5.2	4.6	±0.020	−1.6	±0.026	5.5	*Hd1*
	7	C213	18.6	9.0	±0.016	0.0	±0.022	25.1	*Hd2*
								66.5[h]	
BC_1F_2	3	C515	16.2	4.1	±0.631	0.2	±0.981	38.4	*Hd9*
	3	C1677	5.8	2.9	±0.400	0.0	±0.504	11.7	–
								49.8[h]	
BC_2F_2-3	3	R2404	21.3	6.4	±0.266	−2.0	±0.378	33.3	*Hd6*
	6	E1178SA	18.7	6.5	±0.355	1.8	±0.488	26.3	*Hd3a*
	6	C235	10.7	4.4	±0.244	−1.6	±0.331	12.3	*Hd1*
								76.0[h]	
BC_2F_2-4	3	S1469	15.4	4.8	±0.673	−0.4	±1.013	23.4	–
	4	S10644	3.6	1.3	±1.062	1.7	±1.327	3.8	–
	7	C479	6.2	3.1	±0.760	0.3	±1.016	7.4	*Hd4*
	7	C213	10.6	5.3	±0.863	1.0	±1.148	21.4	*Hd2*
								51.8[h]	
BC_2F_2-5	3	C1677	5.0	1.1	±0.315	−0.4	±0.428	12.1	–
	11	S2137	3.6	0.9	±0.331	0.2	±0.472	8.4	–
	12	R1869	5.5	1.4	±0.560	−0.2	±0.711	13.7	*Hd13*
								44.8[h]	
BC_2F_2-6	3	C515	4.8	2.9	±0.871	0.3	±1.199	8.6	*Hd9*
	6	E1178SA	3.4	2.0	±0.990	1.8	±1.270	5.5	*Hd3a*
	6	C235	11.8	5.1	±0.907	−0.4	±1.244	25.6	*Hd1*
	8	R2976	6.8	3.1	±1.014	−1.5	±1.391	12.5	*Hd5*
								62.7[h]	

[a] LOD thresholds for CIM: F_2, 3.8; BC_1F_2, 4.2; BC_2F_2-3, 2.9; BC_2F_2-4, 3.1; BC_2F_2-5, 2.9; BC_2F_2-6 3.2

[b] Additive effect of the allele from 'Nona Bokra' compared with 'Koshihikari'

[c] Standard error of estimate for additive effect of each QTL

[d] Dominant effect of the allele from 'Nona Bokra' compared with 'Koshihikari'

[e] Standard error of estimate for dominant effect of each QTL

[f] Percentage phenotypic variance explained by each QTL

[g] Chromosomal location of each QTL was compared to 15 QTLs identified in several populations derived from cross between 'Nipponbare' and 'Kasalath' (Yano et al. 2001)

[h] R^2 for the multiple QTL model

in a region of QTLs for heading date reported by Yu et al. (2002) and Thomson et al. (2003).

Six BC_2F_2 populations were also used to detect QTLs for DTH. The average DTH in 2003 for 'Koshihikari' ($n = 10$) was 112 days, whereas no heading was observed in 'Nona Bokra' during the cropping season. The late heading of 'Koshihikari' in this year compared to previous years may be due to slightly lower than average temperature in the summer of 2003 (data not shown). The ranges of DTH in BC_2F_2-1 and BC_2F_2-2 were 108–115 days and 109–115 days, respectively, suggesting that no QTL was involved in variation of DTH in either population (Fig. 2); therefore, we did

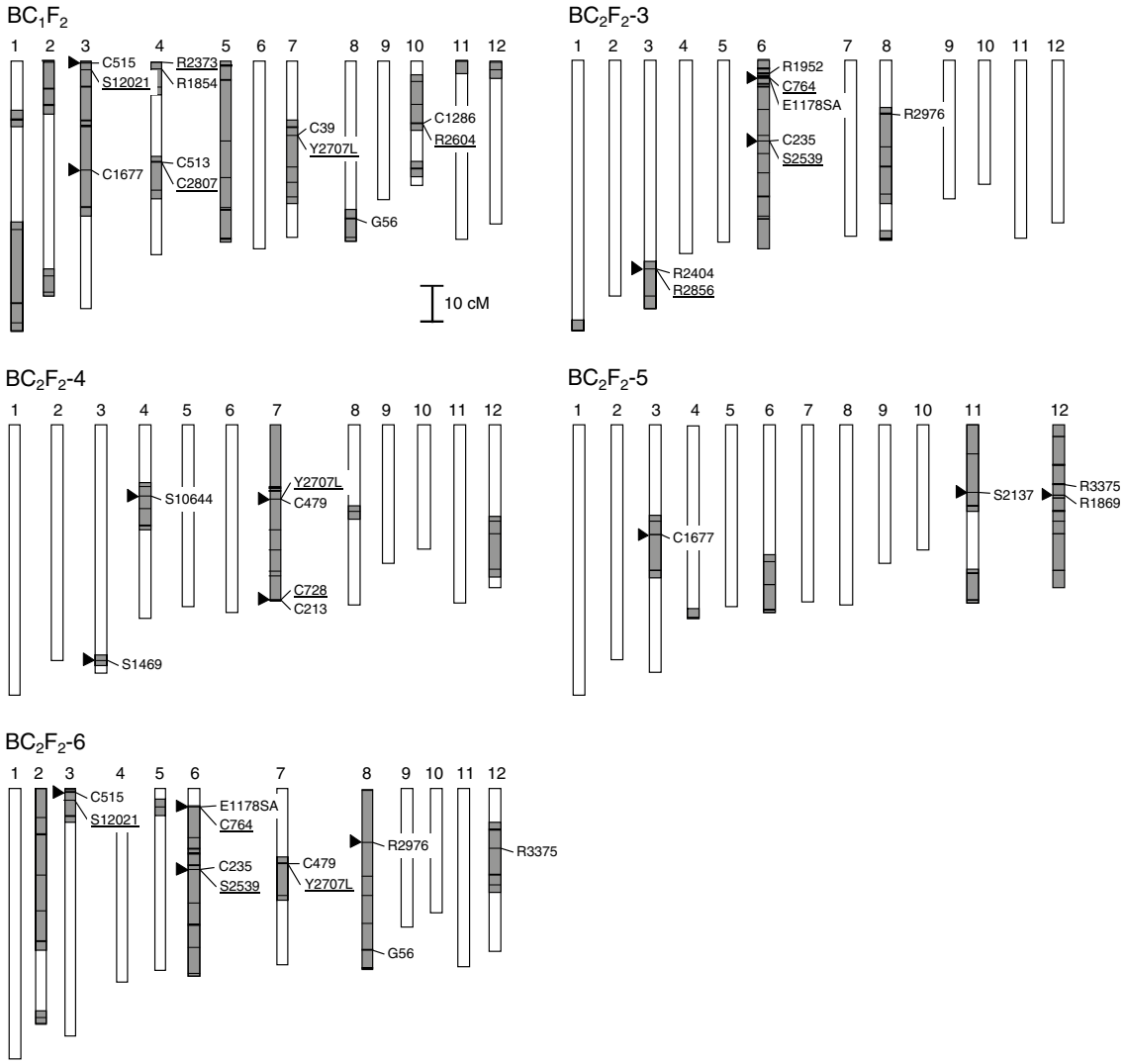

Fig. 3 The positions of QTLs for days to heading in five advanced backcross progeny. Chromosome numbers are indicated above each linkage map. Positions of marker loci are shown as horizontal lines on the framework of the linkage map of 'Nipponbare' × 'Kasalath' constructed by Harushima et al. (1998). *Gray* and *white boxes* represent regions segregated and homozygous, respectively, for 'Koshihikari' alleles. Underlined markers were not mapped in this population but are shown to indicate the positions of nearest markers to *Hd1* to *Hd14*. Nearest markers to *Hd1* to *Hd14* reported previously are as follows; S2539 (*Hd1*), C728 (*Hd2*), C764 (*Hd3a*), Y2707L (*Hd4*), R1952 (*Hd3b*), R2976 (*Hd5*), R2856 (*Hd6*), C560 (*Hd7*), C12534S (*Hd8*), S12021 (*Hd9*), C2807 (*Hd10*), R2373 (*Hd11*), G56 (*Hd12*), R3375 (*Hd13*), and R2604 (*Hd14*). *Arrowheads* indicate the location of markers nearest to the QTLs detected

not carry out QTL analyses of these populations. We did perform QTL analyses of the other four populations, however, which showed wide ranges of phenotypic variation in DTH: 117–158 days in BC_2F_2-3, 114–146 days in BC_2F_2-4, 110–121 days in BC_2F_2-5, and 115–149 days in BC_2F_2-6 (Fig. 2).

Three QTLs for DTH were detected on chromosomes 3 and 6 in the BC_2F_2-3 population (Table 2; Fig. 3). The R^2 ranged from 12.3 to 33.3%. The additive effects of the 'Nona Bokra' alleles of the three QTLs ranged from 4.4 to 6.5 days. Two-way ANOVA revealed that an epistatic interaction occurred between a QTL near R2404 and one near C235 ($P = 0.012$). Based on the chromosomal location, these three QTLs were likely to be *Hd6*, *Hd3a*, and *Hd1*, which were previously detected in the 'Nipponbare' × 'Kasalath' populations (Yano et al. 2001).

Four QTLs for DTH were detected on chromosomes 3, 4, and 7 in the BC_2F_2-4 population (Table 2; Fig. 3). The R^2 ranged from 3.8 to 23.4%. The additive effects of the 'Nona Bokra' alleles of the four QTLs ranged from 1.3 to 5.3 days. Based on the chromosomal location, the QTLs in the vicinity of C479 and C924 on chromosome 7 were likely to be *Hd4* and *Hd2*, respectively. The QTL near S1469 was located in the vicinity of a QTL identified previously in 'Koshihikari' × 'Kasalath' BC_1F_3 populations (Yamamoto et al. 2001).

Three QTLs for DTH were detected on chromosomes 3, 11, and 12 in the BC_2F_2-5 population (Table 2; Fig. 3). The R^2 ranged from 8.4 to 13.7%. The additive effects of the 'Nona Bokra' alleles of the three QTLs ranged from 0.9 to 1.4 days. The QTLs near R1869 and C1677 corresponded with the chromosomal location of *Hd13* (Yano et al. 2001) and QTLs detected by Yu et al. (2002) and Thomson et al. (2003), respectively. The location of another QTL at S2137 did not match the regions of QTLs reported previously in the other populations.

Four QTLs for DTH were detected on chromosomes 3, 6, and 8 in the BC_2F_2-6 population (Table 2; Fig. 3). The R^2 ranged from 5.5 to 25.6%. The additive effects of the 'Nona Bokra' alleles of the four QTLs ranged from 2.0 to 5.1 days. Based on their chromosomal locations, these QTLs were likely to be *Hd9*, *Hd3a*, *Hd1*, and *Hd5*, which were detected in 'Nipponbare' × 'Kasalath' populations (Yano et al. 2001).

Discussion

A wide range of variation in heading date has been observed among rice cultivars (Oka 1958). Many rice cultivars that originated from low-latitude tropical areas tend to exhibit strong PS. Such cultivars are extremely late heading or do not reach the heading stage during the cropping season of Japan, where the day-length from April to August is longer than in tropical regions. The *indica* cultivar 'Nona Bokra' is an extremely late-heading (around 200 DTH) cultivar under the NF and LD (14.5 h light) conditions. Under SD conditions (10 h light), however, it required only about 60 days to flower, about the same as 'Koshihikari', an early-heading *japonica* cultivar, suggesting that 'Nona Bokra' exhibited strong PS.

In this study, we performed genetic analyses of the extremely late heading in 'Nona Bokra' under the NF condition using several types of progeny, including F_2, BC_1F_2, and BC_2F_2 populations, derived from a cross between 'Nona Bokra' and 'Koshihikari'. QTL analyses using these populations detected 12 QTLs for DTH. The additive effects of the 'Nona Bokra' allele at all QTLs increased DTH. When QTLs with large effects segregate simultaneously in the F_2 generation, it is difficult to detect QTLs with small effects (Yano and Sasaki 1997). Therefore, we used advanced backcross progeny BC_1F_2 and BC_2F_2 to reveal minor QTLs responsible for heading date. Eight of the 12 QTLs detected in this study were detected in the analysis of advanced backcross progeny; thus, our findings clearly demonstrate that additional QTLs with relatively small phenotypic effects can be detected by examining advanced backcross progeny.

To detect QTLs for DTH, Ebitani et al. (2005) developed chromosome segment substitution lines (CSSLs), in which a chromosomal segment from 'Kasalath' was substituted into the genetic background of 'Koshihikari'. Their findings showed that CSSLs are a very powerful tool for detecting QTLs with minor phenotypic effects. We are currently developing CSSLs using the 'Koshihikari' genetic background with a substituted chromosome segment from 'Nona Bokra'. Such CSSLs should be very helpful in measuring the DTH of those lines under different day-length conditions to further examine whether the QTLs detected in this study are involved in PS.

We compared the chromosomal locations between the 12 QTLs for DTH revealed in this study and the 15 QTLs (*Hd1–Hd3a*, *Hd3b–Hd14*) for DTH reported in several 'Nipponbare' × 'Kasalath' populations (Yano et al. 2001). Eight of the 12 QTLs were detected near *Hd1*, *Hd2*, *Hd3a*, *Hd4*, *Hd5*, *Hd6*, *Hd9*, and *Hd13* (Figs. 1, 3). Among the other four QTLs, two located on chromosome 3 were identified near regions of QTLs previously detected in other populations (Yamamoto et al. 2001; Yu et al. 2002; Thomson et al. 2003). The remaining two QTLs, located on chromosomes 4 and 11, are likely to be novel. These novel QTLs increased late heading at 'Nona Bokra' alleles but showed very small genetic effects. Using conventional genetics to study DTH, Yokoo et al. (1980) reported that late-heading Japanese cultivars had a late allele of the *Lm* (= *Se1* and *Hd1*) and other late-heading genes. Our study clarified that extremely late heading in 'Nona Bokra' was due to an accumulation of multiple QTLs, including several previously identified QTLs as well as *Hd1*.

Based on their chromosomal location, eight of the QTLs detected in this study may be *Hd1*, *Hd2*, *Hd3a*, *Hd4*, *Hd5*, *Hd6*, *Hd9*, and *Hd13*. These eight QTLs were originally identified in 'Nipponbare' × 'Kasalath' progeny that exhibited early heading under NF conditions in Japan (Yano et al. 1997; Yamamoto et al. 2000; Lin et al. 2002; Monna et al. 2002). Previous studies investigated the response to day-length of eight QTLs (*Hd1*, *Hd2*, *Hd3a*, *Hd3b*, *Hd4*, *Hd5*, *Hd6*, and *Hd9*) using nearly isogenic lines (NILs) with target QTLs under NF, LD, and SD conditions (Yamamoto et al. 2000; Lin et al. 2000, 2002, 2003a; Monna et al. 2002). Their results revealed that *Hd1* and *Hd2* decreased DTH under SD conditions and increased it under LD conditions; *Hd3a* decreased DTH under SD conditions; and *Hd3b*, *Hd4*, *Hd5*, *Hd6*, and *Hd9* increased DTH under LD conditions. Among these QTLs, *Hd1*, *Hd2*, *Hd3a*, *Hd3b*, *Hd5*, and *Hd6* also have been shown to be involved in PS (Lin et al. 2000, 2003a; Yamamoto et al. 2000; Monna et al. 2002).

All QTLs detected in this study increased DTH at 'Nona Bokra' alleles under NF conditions. If these QTLs correspond to the previously identified QTLs for DTH, then

QTLs detected in crosses between the early-heading cultivars 'Nipponbare' and 'Kasalath' would explain the extremely late heading of 'Nona Bokra' under NF conditions. Based on allelic information available for *Hd1*, *Hd3a*, and *Hd6*, we speculated that divergence had occurred between these alleles in 'Nona Bokra' and 'Koshihikari'. For example, the genomic sequence of the 'Koshihikari' *Hd1* allele is the as same as that of 'Nipponbare' (data not shown). The 'Nipponbare' allele of this gene is functional, whereas the 'Kasalath' allele is nonfunctional (Yano et al. 2000). Therefore, 'Nona Bokra' should carry more functional alleles that increase DTH than 'Koshihikari'. In addition, 'Koshihikari' has the same *Hd6* allele as 'Nipponbare' (data not shown). The 'Nipponbare' allele of the gene is nonfunctional, whereas the 'Kasalath' allele is functional (Takahashi et al. 2001). 'Nona Bokra' may have a functional *Hd6* allele that increases DTH, although we were unable to detect which has the more functional allele, 'Nona Bokra' or 'Kasalath'. The difference between DTH in 'Nipponbare' and 'Kasalath' was small (Yano et al. 1997), which may be due to a counterbalance of functional and nonfunctional alleles of genes such as *Hd1* and *Hd6* in each cultivar. In contrast, we suppose 'Nona Bokra' to show extremely late heading compared with 'Koshihikari' by having accumulated alleles of QTLs that increase DTH or enhance PS, regardless of whether they were functional or nonfunctional.

Although these speculations seemed reasonable based on allelic levels, a question arose regarding the QTL detected near *Hd3a* on chromosome 6 in the F_2, BC_2F_2-3, and BC_2F_2-6 populations. This QTL increased DTH at the 'Nona Bokra' allele under NF conditions. In contrast, based on the analysis of a NIL of *Hd3a* (Monna et al. 2002), *Hd3a* did not increase DTH under NF conditions. The *Hd3b* located in the vicinity of the *Hd3a* increases DTH under LD and NF conditions. However, this QTL is not likely to be the same as *Hd3b*, because the chromosomal region around *Hd3b* in the BC_2F_2-6 population was fixed for the 'Koshihikari' allele. Kojima et al. (2002) reported that *Hd3a* encodes an *Arabidopsis* FT-like protein and is involved in the promotion of heading under SD and LD conditions. They also found that an additional *FT*-like gene (designated *RFT1*), which was located 10 kb away from *Hd3a*, was involved in the promotion of heading. To explain this, we propose the following two hypotheses: (1) heading date was delayed as a result of defective function of the *Hd3a* or *RFT1* alleles in 'Nona Bokra', or (2) heading date was delayed due to other, unidentified gene(s). To clarify these hypotheses, it will be necessary to perform further analyses using numerous advanced backcross progeny.

In this study, the QTL with the largest effect on DTH was detected on the distal end of the long arm of chromosome 7 in the F_2 population. In this region, *Hd2* has been mapped using a 'Nipponbare' × 'Kasalath' cross (Yano et al. 1997) and a 'Hayamasari' × 'Italica Livorno' cross (Fujino and Sekiguchi 2005a). Although the molecular cloning of several QTLs, such as *Hd1*, *Hd3a*, *Hd6*, and *Ehd1*, has been achieved, the *Hd2* gene has not been clarified at the molecular level. Map-based cloning of *Hd2* using a 'Nipponbare' × 'Kasalath' cross is still under way. If the QTL on the long arm of chromosome 7 is *Hd2*, then 'Nona Bokra' might carry a novel allele with a different function from those of 'Kasalath' and 'Nipponbare'. Thus, 'Nona Bokra' will be very useful material for the molecular cloning of *Hd2*.

Two-way ANOVAs revealed epistatic interactions between QTLs at R2404 and R1679 in the F_2 population and between QTLs at R2404 and C235 in the BC_2F_2-3 population ($P < 0.05$). Based on their chromosomal locations, these QTLs detected in both populations were consistent with *Hd6* and *Hd1*. In previous studies, epistatic interactions were detected between *Hd1* and *Hd2*, *Hd1* and *Hd3*, *Hd2* and *Hd3*, *Hd2* and *Hd6*, and *Hd1* and *Hd5* (Lin et al. 2000, 2003a; Yamamoto et al. 2000). There is no evidence for the epistatic interaction between *Hd1* and *Hd6*, however, in the previous reports. If the QTLs discovered in this study are in fact *Hd1* and *Hd6*, the epistatic interaction between them represents a novel finding. Among the populations used in this study, there was an inconsistency of additive effects for QTLs near *Hd1* and *Hd3a*. The additive effect of the QTL near *Hd3a* was larger than that of the QTL near *Hd1* in both the F_2 and BC_2F_2-3 populations, but smaller in the BC_2F_2-6 population. This difference cannot be fully explained, although this inconsistency might be due to the influence of the epistatic interaction between *Hd1* and *Hd6*, because the QTL near *Hd6* was detected in both the F_2 and BC_2F_2-3 populations but not in the BC_2F_2-6 population. Further analyses using NILs that combine these QTLs will be required to clarify this inconsistency.

It is difficult to prove an epistatic interaction between two genes in primary populations such as the F_2 generation, in which other related loci segregate simultaneously (Tanksley 1993; Yano and Sasaki 1997); therefore, the one epistatic interaction between previously identified QTLs was barely detectable in our F_2 population. In the advanced backcross progeny (BC_2F_2 populations) used in this study, one or two chromosomes were heterozygous, whereas nearly all other regions were homozygous for 'Koshihikari' alleles. These populations were selected to dissect minor QTLs responsible for DTH, whereas previous studies developed advanced backcross progeny of 'Nipponbare' × 'Kasalath' to investigate epistatic interaction between two QTLs detected in the primary populations (Lin et al. 2000, 2003a; Yamamoto et al. 2000). Thus, our populations likely were not adequate for the evaluation of epistatic interaction

between previously identified QTLs, and the contribution of the epistatic interaction between QTLs to the extremely late heading of 'Nona Bokra' could not be clarified at the genetic level. However, the variation of heading date in each population was explained well by the accumulation of additive effects of the QTLs detected in them. In particular, additive effects of QTLs detected near previously identified QTLs responsible for DTH accounted for a large proportion of the phenotypic variation in each BC_2F_2 population. Thus, our findings suggest that the extremely late heading of 'Nona Bokra' was generated predominantly by the accumulation of additive effects of QTLs rather than by epistatic interactions between QTLs.

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