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Identification of quantitative trait loci controlling heading date in rice using a high-density linkage map

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Abstract Quantitative trait locus (QTL) analysis has been carried out to identify genes conferring heading date in rice. One hundred and eighty six F_2 plants derived from a cross between a *japonica* variety, Nipponbare, and an *indica* variety, Kasalath, were used as a segregating population for QTL mapping and more than 850 markers were employed to identify QTLs. Scan-analysis revealed the existence of two QTLs with large effects, *Hd-1* and *Hd-2*, one in the middle of chromosome 6 and one at the end of chromosome 7, respectively. For both loci, the Kasalath alleles reduced days-to-heading. In addition, three QTLs with minor effects, *Hd-3*, *Hd-4* and *Hd-5*, were found to be located on chromosomes 6, 7 and 8 based on a secondary scan analysis which was carried out by removing the phenotypic effects of *Hd-1* and *Hd-2*. For the three secondary loci, the Nipponbare alleles reduced days-to-heading. The five QTLs explained 84% of the total phenotypic variation in the F_2 population based on a multiple-QTL model. The presence of a digenic interaction between *Hd-1* and *Hd-2* was clearly suggested.

Key words RFLP markers · Days-to-heading · QTL analysis · Rice · Epistatic interaction

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Introduction

Heading date is one of the most important traits for the adaptation of rice to different cultivation areas and crop seasons. Control of heading date is therefore a major objective in rice breeding programs. Large variation in heading date has been found among cultivated varieties of rice (Vergara and Chang 1985). Many researchers have carried out genetic analysis for heading date (Yokoo et al. 1980; Yamagata et al. 1986; Poonyarit et al. 1989). Heading date in rice is basically determined by two factors, duration of the basic vegetative growth (BVG) and photoperiod-sensitivity (PS). Several genes are involved in controlling these two factors. Chang et al. (1969) reported that BVG was controlled by two or three genes. *Ef-1* is a major gene that controls the duration of BVG and is located on chromosome 10 (Sato et al. 1988). PS is also controlled by several genes including *Se-1*(*Lm*), *Se-3*, *Se-4*, *Se-5*, *Se-6*, *Se-7*, *E1*, *E2* and *E3* (Yokoo et al. 1980; Yamagata et al. 1986; Poonyarit et al. 1989; Ohshima et al. 1993; Yokoo and Okuno 1993; Tsai 1995). Yokoo et al. (1980) reported that the *Se-1* gene is closely linked to the blast-resistance gene *Pi-z*^{t} on chromosome 6. Recently, molecular markers linked to *Se-1* have also been identified (Mackill et al. 1993). However, most of the allelic relationships among these genes and their chromosomal locations remain uncertain, because it is very difficult to conduct genetic analysis of variation in heading date due to the number of genes involved.

The development of molecular markers (e.g. restriction fragment length polymorphism) makes it possible to identify individual loci controlling quantitative traits (Tanksley 1993). Using molecular markers, a number of studies have been carried out to identify the genetic nature of quantitative traits in several crop plants such as tomato (Paterson et al. 1988; DeVicente and Tanksley 1993) and maize (Edwards et al. 1992; Stuber et al. 1992).

In rice, four independent linkage maps have been constructed using molecular markers (McCouch et al. 1988; Saito et al. 1991; Causse et al. 1994; Kurata et al. 1994). Kurata et al. (1994) developed a high-resolution and a high-density linkage map with more than 1300 DNA markers. Quantitative trait locus (QTL) analyses using molecular markers have also been carried out for partial resistance to blast disease (Wang et al. 1994), kernel elongation on cooking (Ahn et al. 1993), several morphological traits (Xiao et al. 1996), and heterosis (Xiao et al. 1995). In the present study, in order to facilitate the genetic analysis of heading date and the positional cloning of the genes involved, we have used the densely mapped DNA markers to genetically dissect phenotypic differences for heading date in the F_2 population which was also used for the construction of the high-density linkage map (Kurata et al. 1994).

Materials and methods

Plant materials

A *japonica* variety, Nipponbare, was crossed with an *indica* variety, Kasalath. 186 F_2 individuals, derived from an F_1 plant by self pollination, were used in this study. Nipponbare is photoperiod-sensitive. No prior information was available on the response of Kasalath to photoperiod. F_2 individuals and their parental lines were cultivated at Hokuriku National Agricultural Experiment Station, Joetsu, Niigata, Japan (37°N latitude). Seeds were sown on 10 April 1990 and were transplanted on 20 May in an irrigated rice field. Plants were grown under natural day length conditions and the heading date of each F_2 plant was parental plants was monitored for the appearance of the first panicle. Leaf material for DNA extraction was collected after monitoring heading date to minimize damage to F_2 plants. Variation in heading date was expressed as the number of days from seeding to heading.

RFLP data analysis

The F_2 population used in this analysis was the same population that was used to develop the high-density RFLP and RAPD linkage map of rice with 1383 DNA markers (Kurata et al. 1994). Segregation data for each F_2 individual (total 186) and for all the DNA markers (total 1383) were available for QTL analysis. Kurata et al. (1994) suggested that this linkage map appears to cover most of the genome, based on the fact that very few markers extended the size of the map length after mapping the first 1000 markers. All experimental procedures for DNA extraction, Southern blotting, hybridization and linkage-map construction have been described previously by Kurata et al. (1994). Two micrograms of total DNA of each F_2 plant was digested with eight kinds of restriction enzymes, *Bam*HI, *Bgl*II, *Eco*RV, *Hin*dIII, *Apa*I, *Dra*I, *Eco*RI and *Kpn*I. The digested DNAs were blotted onto a nylon membrane. Southern hybridization and detection were carried out using the ECL direct labelling and detection system (Amersham).

QTL analysis

For QTL analysis we selected 857 loci, which cover the whole of the rice genome, from the total of 1383 RFLP and RAPD markers (Kurata et al. 1994). When multiple markers including RFLPs and RAPDs were located at the same position on the linkage map, one representative marker, usually a co-dominant RFLP marker, was selected to increase the accuracy of QTL analysis. Of all markers selected, 717 (about 83%) were co-dominant. A framework linkage map for QTL analysis was reconstructed using genotype data of selected markers with MAPMAKER/EXP ver 3.0 (Lander et al. 1987). The average genetic distance between markers is about 1.8 cM. Most of the intervals between markers on the map (more than 600) are less than 2 cM. The map locations of putative QTLs were determined by interval mapping using MAPMAKER/QTL ver.1.1 (Lander and Botstein 1989; Lincoln et al. 1992). QTL investigation was carried out in several steps. In the first examination, if the LOD score exceeded the threshold, the position with the highest LOD score on each chromosome was estimated as the most likely position of the QTL based on a single-QTL model. A LOD score of 3.0 was used as the threshold during the first examination to avoid false positives. After QTLs were detected in the first examination, a second examination for other QTLs was carried out. The second examination removed the effect of the major QTLs revealed during the first examination using the "sequence" command of MAPMAKER/QTL (Paterson et al. 1991). In the second examination, if the LOD score exceeded the score of identified QTL(s) in first examination by a value of 3.0, other putative QTLs were identified based on a multiple-QTL model. All analyses used map intervals of 2.0 cM or less. In MAPMAKER/QTL, primary QTL analysis was carried out based on the condition of "unconstrained genetics", in which two independent parameters [additive effect (a) and dominant effect (d)] were used for testing the QTLs. To identify the mode of inheritance, putative QTL regions were re-examined out by three constrained genetic tests, such as "dominance", "recessive" and "additive", using MAPMAKER/QTL. These analyses were carried out by forcing $d = 0$ in the "additive mode", $d = a$ in the "dominance mode" and $d = -a$ in the "recessive mode" (Lincoln et al. 1992). Tests for the statistical reliability of the estimated modes of inheritance were done according to Paterson et al (1991). Hence, a 1-LOD reduction in likelihood has been used as a criterion for ruling out the mode of inheritance. The phenotypic effects, such as the additive effects (a), the dominance effects (d) and the percent of total phenotypic variation explained (PVE) by each putative QTL, were also estimated by MAPMAKER/QTL. To evaluate digenic interaction between pairs of QTLs, an analysis of variance for each genotypic class, represented by the genotypes of the nearest marker loci, was conducted using the SAS GLM PROC (SAS Institute 1989).

Results

Phenotypic variation

The frequency distributions of days-to-heading of the F_2 population and their parental lines, Nipponbare and Kasalath, are shown in Fig. 1. The mean value of days-to-heading for Nipponbare was 122 days (range 121–125 days), while that of Kasalath was 117 days (range 115–119 days). The difference between the number of days-to-heading of these parental lines was small. However, the variation for days-to-heading of their F_2 progeny was large (range 104–164 days) and continuous. The frequency distribution of days-to-heading suggested an underlying bimodal distribution and showed transgressive segregation. A number of F_2 plants with a very late heading phenotype was observed. These results suggested that genes with relatively large effects and genes with effects opposite to those predicted based on the phenotypes of parents

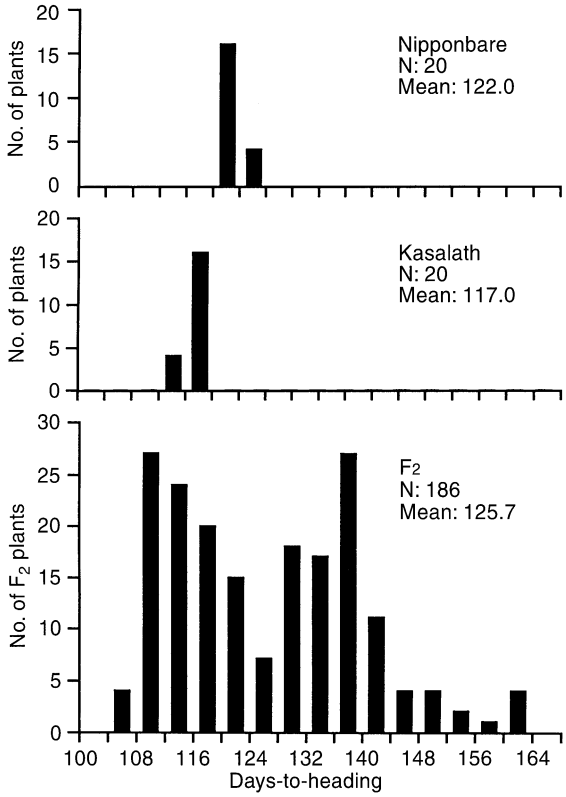

Fig. 1 Frequency distributions of days-to-heading in F_2 population and its parental lines, Nipponbare and Kasalath

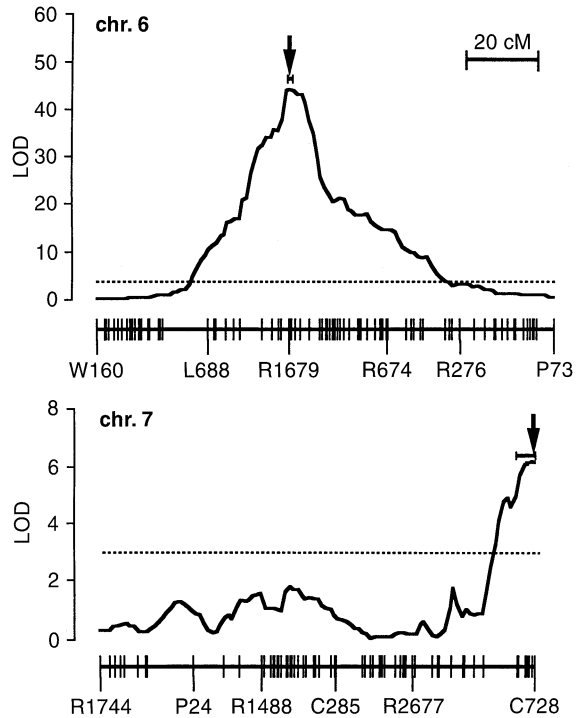

Fig. 2 QTL likelihood curves of the LOD score of heading date for chromosomes 7 and 8 in the first examination. The RFLP linkage maps of each chromosome are presented at the bottom of each figure together with the nearest markers for putative QTLs. Some other RFLP (*C, R, L and TEL*) and RAPD (*P*) markers, including markers at the most distal end of the maps, were also added in order to provide an indication of the orientation of the linkage maps. The linkage map of the distal end of chromosome 7 has been changed due to data corrections (Harushima et al. 1995). A genetic distance of 20-cM (Kosambi scale) is shown in the figure. The *dotted horizontal line* indicates the significant threshold of LOD score 3.0 to detect putative QTLs. The likelihood position of putative QTLs are the peak of the curve indicated by the *arrow*. The *bar under arrow* indicates a 1-LOD support confidence interval for putative QTLs

were involved in the segregation of heading date in this cross.

QTLs controlling heading date

In the first examination, two distinct loci with relatively large effects were detected. One QTL peak for *Hd-1* (LOD = 44.2) was found to be located exactly at marker R1679 on chromosome 6. The highest value of the LOD curve for another QTL, *Hd-2* (LOD = 6.2), appeared to be exactly at marker C728, which is on the distal end of chromosome 7 (Fig. 2, Table 1). There was no direct evidence for the complete coverage of the linkage map for chromosome 7. However, Kurata et al. (1994) have already indicated good genome coverage of the linkage map. Moreover, even though more than 2000 markers mapped to the linkage map, no additional marker was mapped at the distal side from C728 (unpublished data). Based on this situation, it was considered that C728 can be treated as a peak position of the LOD curve for *Hd-2*. For both loci, the Kasalath alleles reduced days-to-heading. Based on a LOD reduction criterion, an "additive" mode of inheritance was most likely for *Hd-1* and "additive" was also more likely, though "recessive" could not be ruled out, for *Hd-2* (Table 1). In the single-QTL model, estimated additive effects of the Kasalath allele for *Hd-1* and *Hd-2* were -14.5 days and -7 days, respectively. These two QTLs explained about 67% and 14% of the total phenotypic variation in the F_2 population.

In the second examination, by removing the phenotypic effects of the two QTLs detected in the first analysis, three other putative QTLs, *Hd-3*, *Hd-4* and *Hd-5*, with relatively small effects, were identified on chromosomes 6, 7 and 8. The two peak positions for *Hd-3* and *Hd-4*, were found to be located exactly at marker C226A on chromosome 6 and marker L538T3 on chromosome 7, respectively. *Hd-5* was mapped in the R2736–R902 marker interval on chromosome 8 (Fig. 3, Table 1). For these three loci, the Nipponbare alleles reduced days-to-heading. The estimated additive effects of the Kasalath allele for *Hd-3*, *Hd-4* and *Hd-5*, were 2.1 days, 2.9 days and 3.4 days, respectively (Table 1). For the mode of inheritance of the these three putative QTLs, "recessive" was more likely but

Table 1 Putative QTLs controlling heading date detected during the first and second examination on the multiple-QTL model. NML: the nearest marker locus to the QTL, figures in parenthesis indicate the genetic distance (cM) between the peak position of the LOD curve and NML. LOD: log-likelihood value calculated by MAPMARKER/QTL ver.1.1 in the condition of unconstrained genetics. *a*: additive effect of the Kasalath allele on days-to-heading. *d*: dominant effect of the Kasalath allele. *d*/*a*: degree of dominance. PVE: percent of total phenotypic variance explained by the QTL. MOI: mode of inheritance estimated based on an analysis with constrained genetics, a 1-LOD reduction in likelihood of three genetic models, additive (A), dominance (D) and recessive (R), has been used as a criterion for ruling out the mode of inheritance. The first letter indicates the most likely mode and the second letter indicates a mode which could not be ruled out. Thus, AR indicates that "additive" is more likely but "recessive" is not ruled out. In *Hd-3*, *Hd-4* and *Hd-5*, all calculated and estimated values are based on the analysis of a multiple-QTL model with *Hd-1* and *Hd-2*. The significant threshold in the secondary analysis was a LOD score of 63.6

QTL	Chromosome	NML	LOD	Effects on the phenotype				MOI
				a	*d*	*d*/*a*	PVE	
Hd-1	6	R1679 (0)	44.2	− 14.5	2.3	− 0.16	66.7	A
Hd-2	7	C728 (0)	6.2	− 7	2.8	− 0.4	14.2	AR
Hd-3	6	C226A (0)	64.4	2.1	− 1.9	− 0.90	79.9	RA
Hd-4	7	L538T3 (0)	65.3	2.9	− 1.9	− 0.66	80.4	RA
Hd-5	8	R902 (1.4)	65.7	3.4	0.9	0.26	80.6	AD

"additive" could not be ruled out for *Hd-3* and *Hd-4*. On the other hand, "additive" was more likely, though "dominance" could not be ruled out, for *Hd-5* (Table 1). The five QTLs detected explained about 84.3% the of total phenotypic variation in the F_2 population.

Using the estimated additive effects of the Kasalath alleles on the five QTLs, we predicted a difference of about 26 days-to-heading between the two parental lines, Nipponbare and Kasalath, based on a simple additive model (two-times the sum of the additive effects). However, the observed difference was only 5 days (Fig. 1). Although the estimated additive effects of the five QTLs might not be precise, due to the relatively small population size (186 plants) employed in this study, this discrepancy might also be caused by specific interactions among the detected QTLs or by the existence of other undetected QTLs.

To detect the existence of gene interactions, genotype data of the nearest marker loci for each QTLs were used for an analysis of variance of the classified genotype classes. Based on a two-way ANOVA, significant interactions were only detected in comparisons involving *Hd-1*; interactions between the other loci were difficult to detect because of the large effect of *Hd-1*. Differences among mean values of days-to-heading in the possible genotype classes for the pairs of *Hd-1* and the other detected QTLs are shown in Fig. 4. Digenic interaction between *Hd-1* (represented as R1679) and *Hd-2* (C728) was significant ($P < 0.0001$). There was no large effect of *Hd-2* in the sub-population homozygous for the Kasalath allele of *Hd-1*, whilst the effect of *Hd-2* in the sub-population heterozygous and homozygous for the Nipponbare allele of *Hd-1* was relatively large. On the other hand, for the combinations of *Hd-1* with *Hd-3* (C226A), *Hd-4* (L538T3) and *Hd-5* (R902), two-way ANOVA results did not indicate the existence of specific interactions ($P = 0.260$, $P = 0.053$ and $P = 0.150$, respectively). However, in the sub-population of homozygotes for the Nipponbare allele of *Hd-1*, a large difference for days-to-heading was observed among the genotype classes of *Hd-3* and *Hd-4* compared to those of the other two sub-populations of heterozygotes and homozygotes for the Kasalath allele of *Hd-1*. In the combination of *Hd-1* and *Hd-4*, the result was very close to significance ($P = 0.053$), suggesting that there might be a specific interaction between *Hd-1* and *Hd-4*. Based on this study, it was difficult to clarify the interaction between these QTLs. Further analysis will be necessary to provide a precise estimation of the interactions.

Discussion

Genetic analyses of heading date in rice have been carried out by many researchers. Previous studies in rice revealed the existence of several dominant genes controlling heading date, such as *Se-1, Se-3, Se-4, Se-5, Se-6, Se-7, E1, E2* and *E3* for PS and *Ef-1* for BVG (Yokoo et al. 1980; Ohshima et al. 1993; Yamagata et al. 1986; Sato et al. 1988; Yokoo and Okuno 1993; Tsai 1995). Other studies revealed that two modifier genes, *i-Se-1* and *En-Se-1*, control heading date (Chang et al. 1969; Sano 1992). However, the chromosomal locations of the several genes for PS and for BVG still remain to be determined. For the genetic analysis of naturally occurring variation for heading date, segregating populations usually show continuous variation caused by multiple gene action. Therefore, it was necessary to use well-characterized genetic stocks, such as a near-isogenic lines, for precise linkage mapping. For these reasons, it is rather difficult to map genes for PS and for BVG on the linkage map in which morphological and physiological markers have been

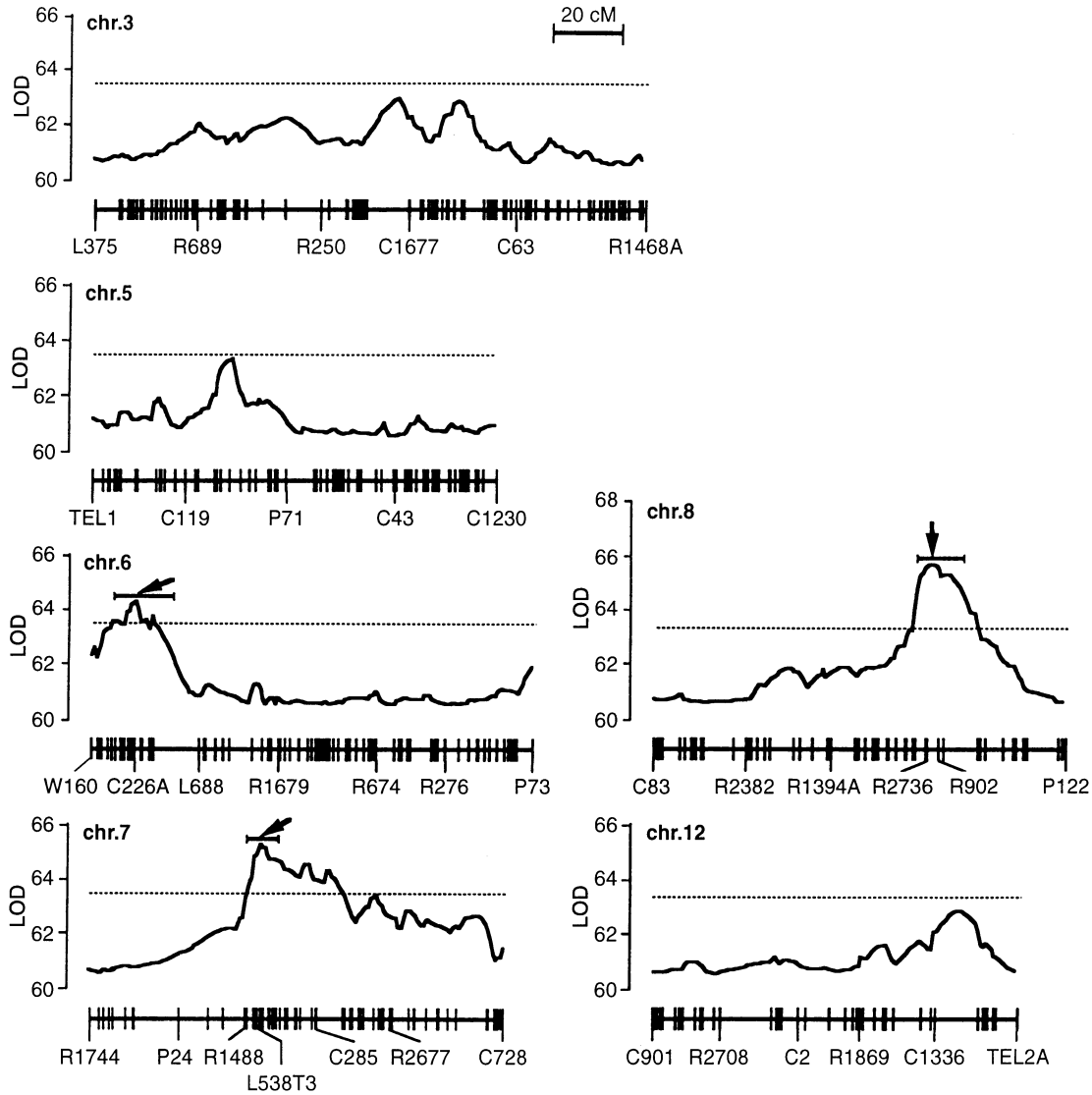

Fig. 3 QTL likelihood curves of the LOD score of heading date for six chromosomes in the second examination, which was carried out by removing the phenotypic effects of the QTLs, *Hd-1* and *Hd-2*, detected during first examination. The *dotted horizontal line* indicates a LOD score of 63.6, which is the significant threshold to detect additional putative loci in the multiple-QTL model. The other information is the same as in Fig. 2

used. In the present study, we employed QTL analysis to determine the chromosomal location of genes conferring heading date in rice. As a result, at least five loci have been identified.

Beavis et al. (1991) hypothesized that some qualitative trait loci, such as dwarfing genes, could be quantitative trait loci in maize. Our observations support this hypothesis for heading date in rice. The major photoperiod sensitivity gene, *Se-1*, has been found to be located on chromosome 6 and closely linked to an isozyme marker, Phosphoglucose isomerase-2 (*Pgi-2*) (Kinoshita 1995), and a blast resistance gene, *Pi-z*^{t} (Yokoo et al. 1980). In our study, *Hd-1*, with a large effect on heading date was found to be located in the middle of chromosome 6. A cDNA clone coding for phosphoglucose isomerase (V19B) was also mapped in the vicinity of R1679 which is closely linked to *Hd-1*. (Kurata et al. 1994). According to the location of both loci on chromosome 6, it is considered that *Hd-1* might be the same locus as *Se-1*. Sano (1992) revealed that the strong photoperiod sensitivity in one accession of *Oryza rufipogon*, W593, is controlled by *Se-1* and its dominant enhancer, *En-Se-1*. The gene, *En-Se-1*, was found to be located on chromosome 6 and linked to the *wx* locus. In the present study, *Hd-3* was located at marker C226A, also linked to *wx* on chromosome 6. The location of the gene *En-Se-1* also showed a good correspondence to that of *Hd-3*. Moreover, one of photoperiod-sensitivity genes, *E1*, was found to be located on chromosome 7 and was linked to the morphological marker, *Rc* (Tsai 1976; Okumoto et al. 1992). In our study, the marker L538T3 linked to *Hd-4*

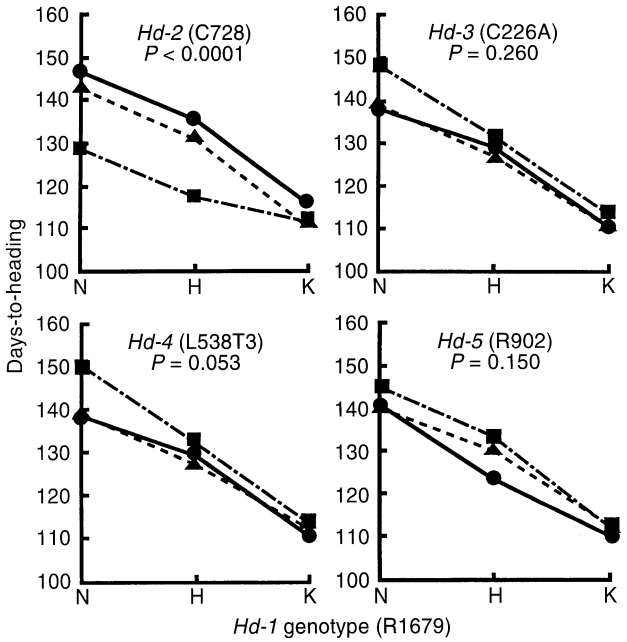

Fig. 4 Differences in mean values for days-to-heading in different combinations of genotype classes between *Hd-1* and *Hd-2*, *Hd-3*, *Hd-4* and *Hd-5*. Genotype data of the nearest RFLP marker loci were used (as that of five detected QTLs). The probabilities (*P*) of testing the hypothesis (that the gene effects of combining two loci is additive), which were calculated in a two-way ANOVA, are given in each figure. *N*, *H and K* indicate homozyguosity for the Nipponbare allele, and heterozyguosity and homozyguosity for the Kasalath allele at *Hd-1*, respectively. The genotypes of *Hd-2*, *Hd-3*, *Hd-4* and *Hd-5* are shown in each figure as homozygous Nipponbare (●), heterozygous (▲) and homozygous Kasalath (■), respectively

was also found to be located in the vicinity of *Rc* on chromosome 7 (Lin et al. 1994). The chromosomal locations of several previously reported genes, such as *E2*, *E3*, *Se-4*, *Se-6* and *Se-7*, are not yet known. *Hd-2* on chromosome 7 and *Hd-5* on chromosome 8, detected in the present study, might correspond to some of these known genes. In our study, only the map locations of QTLs for heading date were clearly determined. Although further analysis will be necessary to clarify the allelic relationship between known genes and the QTLs identified in this paper, the results suggested that QTL mapping will be a powerful method to facilitate the analysis of allelic relationship among genes controlling heading date. A comparison of these QTLs with these known genes will also be required to clarify the function of the QTLs, i.e. photoperiod-sensitivity and basic vegetative growth. The development of near-isogenic lines will be useful for a precision-mapping and characterization of detected QTLs (Yano and Sasaki 1997).

The relationship between the estimated effects of QTLs identified in the F_2 population and the observed difference between the parents is difficult to account for. Part of this discrepancy might be explained by an imprecise estimation of gene action in *Hd-2* and *Hd-4* due to epistatic interations between *Hd-1* and *Hd-2* and *Hd-4*. The additive effect of the Kasalath allele of *Hd-4* was estimated at 2.9 days in the linear model (i.e. no interaction). However, by considering two-locus interaction between *Hd-1* and *Hd-4*, the effects of *Hd-4* might be larger. Another explanation for the discrepancy might be the failure to detect additional QTLs responsible for heading date. In the present study, a relatively high LOD threshold of 3.0 was used to identify putative QTLs in order to avoid false positives. In the secondary examination for identifying QTLs (Fig. 3) there were several peaks on the LOD curve, whose values were just below the threshold (for example, chromosomes 3, 5 and 12). It was not possible to prove the existence of QTLs at these locations given their small effects. Further QTL analysis will probably require near-isogenic lines of these putative chromosomal regions.

In this analysis, more than 850 independent markers, most of which were co-dominant, have been used to detect putative QTLs, and the mean genetic distance of marker intervals was shown to be less than 1.8 cM. As a result, the average interval between markers in the vicinity of LOD peak scores, was 1.0 cM (data not shown), and most likely the position of putative QTLs was determined at the marker site (Table 1). Densely mapped markers also enabled us to detect putative QTLs with small effects (*Hd-3*, *Hd-4* and *Hd-5*). However, even using a large number of genetic markers, it has been difficult to determine the precise locations and gene actions of individual QTLs in the primary analysis. To achieve high-resolution mapping of QTLs in tomato, overlapping substitution lines have been employed (Paterson et al. 1990). The development of such new genetic stocks will be necessary to carry out further analysis, such as precision mapping, of the QTLs detected in our study.

The genetic analysis of quantitative traits using molecular markers also makes it possible to evaluate epistatic interactions between individual loci. Digenic interactions among detected QTLs have also been estimated in this study. A strong interaction between *Hd-1* and *Hd-2* was clearly suggested and the presence of interactions between *Hd-1* and *Hd-4* could not be completely ruled out. Tanksley (1993) indicated that these results might be due to statistical limitations, such as the relatively small population size and the number of markers used in the QTL analysis, for the detection of interacting QTLs. Even using densely mapped markers in this analysis, it is difficult to detect QTLs with specific interactions due to the small size of the population (186 F_2 plants). As Tanksley (1993) pointed out, the development of near-isogenic lines for single and multiple QTLs will be necessary to clarify the real nature of epistatic interactions. To verify the suggested digenic interactions among QTLs, near-isogenic lines for five QTLs are currently being developed using marker-aided selection (Yano and Sasaki 1997).

The control of heading date is an important objective in rice breeding, because it is a major determinant of the regional and seasonal adaptation of rice varieties. Understanding the individual genetic factors involved in heading date would be very useful for rice breeding. This would facilitate the manipulation of heading date using genes with relatively minor effects. However, it is obvious that the QTLs detected depend on the cross combination employed. To find the common loci controlling heading date across many cross combinations is a most important aspect of rice breeding. In the present study, we have detected at least five QTLs responsible for heading date using only one cross combination. Li et al. (1995) have also identified five putative loci for heading date on chromosomes 3, 8 and 9 using one *indica/japonica* cross. Xiao et al. (1995,1996) reported that QTLs for days-to-heading mapped on chromosomes 3, 4, 7, 8 and 11 using *indica/japonica* crosses. Of these, the location of QTLs on chromosome 7 and 8, which had relatively large effects, showed a good correspondence with those of *Hd-4* and *Hd-5* detected in this study. However, two QTLs with relatively large effects, which were detected on chromosome 6 and at the end of chromosome 7 in our study, were not found previously. On the other hand, no QTLs were detected by us on chromosome 3. Based on the comparison of this and previous studies, there are at least eight or nine loci which control heading date in rice. Further QTL analyses using different cross combinations may be necessary to identify the full set of genes controlling heading date in rice. Identification of most of the putative QTLs for heading date would make it easier to manipulate this trait.

Genes controlling heading date are also involved in the transition of rice from the vegetative phase to the reproductive phase. The identified QTLs in the present study will be very useful for the further analysis of the biochemical and physiological basis of heading in rice. The isolation of genes involving in heading date should lead to an eventual understanding of these phenomena. Recently, a photoperiod sensitivity gene, *CO*, has been isolated in *Arabidopsis* by positional cloning (Putterill et al. 1995). Positional cloning will be one strategy for the isolation of genes detected in the present study. Several near-isogenic lines which carry the Kasalath alleles for each QTL in the genetic background of Nipponbare are currently being developed using marker-aided selection (Yano and Sasaki 1997). Once the near-isogenic lines for QTLs become available, it will be possible to deal with putative QTLs as single Mendelian factors and to map the QTLs more precisely. These QTLs can then be isolated by the positional-cloning method.

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