Theor Appl Genet (1998) 97 : 37–44

T. Yamamoto · Y. Kuboki · S. Y. Lin
T. Sasaki · M. Yano

Fine mapping of quantitative trait loci *Hd-1*, *Hd-2* and *Hd-3*, controlling heading date of rice, as single Mendelian factors

Received: 5 November 1997 / Accepted: 10 February 1998

Abstract Fine mapping was carried out on three putative QTLs (tentatively designated as *Hd-1* to *Hd-3*) of five such QTLs controlling heading date in rice that had been earlier identified using an F_2 population derived from a cross between a *japonica* variety, 'Nipponbare', and an *indica* variety, 'Kasalath', using progeny backcrossed with 'Nipponbare' as the recurrent parent. One BC_3F_2 and two BC_3F_1 plants, in which the target QTL regions were heterozygous and most other chromosomal regions were homozygous for the 'Nipponbare' allele, were selected as the experimental material. Self-pollinated progeny (BC_3F_2 and BC_3F_3) of the BC_3F_1 or BC_3F_2 showed continuous variation in days to heading. By means of progeny testing based on BC_3F_3 or BC_3F_4 lines, we determined the genotypes of each BC_3F_2 or BC_3F_3 individual at target QTLs. Their segregation patterns fitted Mendelian inheritance ratios. When the results obtained by RFLP analysis and progeny tests were combined, *Hd-1*, *Hd-2* and *Hd-3* were mapped precisely on chromosomes 6, 7 and 6, respectively, of a rice RFLP linkage map. The results demonstrated that QTLs can be treated as Mendelian factors. Moreover, these precise locations were in good agreement with the regions estimated by QTL analysis of the initial F_2 population, demonstrating the high reliability of QTL mapping using a high-density linkage map.

Key words *Oryza sativa* L. · RFLP mapping · QTL · Heading date · Backcross progeny

Communicated by J. W. Snape

T. Yamamoto[1] · Y. Kuboki · S. Y. Lin
Rice Genome Research Program, Institute of Society for Techno-innovation of Agriculture, Forestry and Fisheries (STAFF), Tsukuba, Ibaraki 305, Japan

T. Sasaki · M. Yano (✉)
Rice Genome Research Program, National Institute of Agrobiological Resources (NIAR), 2-1-2, Kannondai, Tsukuba, Ibaraki 305, Japan
Fax: 0298-38-7468
E-mail: myano@abr.affrc.go.jp

Present address:
[1] Plant Breeding and Genetics Research Laboratory, Japan Tobacco, 700 Higashibara, Toyoda, Iwata, Shizuoka 438, Japan

Introduction

Heading date in crop species is one of the major determinants of adaptation to different cultivation areas. It is closely related to the transition from vegetative growth to reproductive growth and is affected by several environmental conditions, such as photoperiod, temperature and light intensity. From the standpoints of not only biological interest but also breeding strategies, it is important to understand the genetic basis of heading date.

Genetic analyses of heading date in rice have been carried out by many researchers. There are several major genes affecting heading date, that relate to either of two factors: basic vegetative growth or photoperiod sensitivity. The chromosomal locations of some of these genes have already been clarified using morphological markers or DNA markers (Yokoo et al. 1980; Sato et al. 1988; Oosumi et al. 1989; Sano 1992; Mackill et al. 1993; Yokoo and Okuno 1993). However, statistical analyses have indicated that several genes participate in the variation for heading. Since the strategy of genetic analysis of quantitative trait loci (QTL analysis) using molecular markers was established (Paterson et al. 1988; Tanksley 1993), many chromosomal regions affecting rice heading date have been reported (Xiao et al. 1995; Li et al. 1995; Xiao et al. 1996; Lin et al. 1996; Yano et al. 1997; Lu et al. 1997).

Although many reports have shown putative chromosomal regions of QTLs to be controlling various agronomic traits, two basic questions about QTL analysis still remain (Paterson et al. 1988; Tanksley 1993; Yano and Sasaki 1997): (1) whether the QTL corresponds to one locus only or to a chromosomal region containing a cluster of genes, each with relatively small genetic effects; and (2) whether enough reliability for detailed mapping can be obtained by QTL analysis. These problems have to be solved when we embark on marker-assisted selection (MAS) or map-based cloning of genes at QTLs. Thus, it is necessary to deal with each QTL as a single Mendelian factor and to localize each QTL precisely on the linkage map (fine mapping). However, the populations previously used in QTL analyses of rice were mainly F_2 s, BC_1F_1 s, recombinant inbred lines or doubled haploid lines (McCouch and Doerge 1995; Yano and Sasaki 1997). These populations, which segregate multiple genetic factors on the whole genome simultaneously, cannot be used for the precise mapping of one of multiple QTLs. Moreover, determination of the true genetic action of the QTL would be more difficult, because the genetic parameters of a given QTL are often affected by the segregation of other QTLs.

In plant genetics, near-isogenic lines (NILs) developed by backcrossing have been widely used to perform accurate genetic analysis because the segregating populations obtained from crossing NILs and their recurrent parents simplify genetic variations by excluding extra genetic factors. This type of QTL analysis has been employed for the mapping of the time of ear emergence in wheat, and inter-varietal substitution lines were used as kind of NILs (Law 1966). After Tanksley (1993) systematized this concept by using molecular markers, two major QTLs controlling morphological differences between maize and teosinte were separated as single Mendelian loci (Dorweiler et al. 1993; Doebley et al. 1995). Alpert and Tanksley (1996) also indicated that a major QTL for fruit weight in tomato was a single gene by demonstrating that a single large DNA fragment (150 kb) contained the QTL. Since most quantitative variations are controlled by combinations of a few major QTLs and many minor QTLs distributed throughout the whole genome (Tanksley 1993; Yano and Sasaki 1997), it will also be very important to deal with the minor QTLs as single genes.

Yano et al. (1997) reported that five putative QTLs, *Hd-1*, *Hd-2*, *Hd-3*, *Hd-4* and *Hd-5*, controlled heading date in the F_2 population between a *japonica* rice variety, 'Nipponbare', and an *indica* rice variety, 'Kasalath'. *Hd-1*, *Hd-2* and *Hd-3* were estimated to be located on the centromeric region of chromosome 6, on the distal end of chromosome 7 and on the distal region of chromosome 6, respectively. In the study presented here, we selected desirable backcrossed plants, in which only target QTLs would be segregated as the main genetic factors, by a whole genome survey using restriction fragment length polymorphism (RFLP) markers. Successively, we tried to locate on the RFLP linkage map not only the two major QTLs (*Hd-1* and *Hd-2*) but also one of the minor QTLs (*Hd-3*), as the first step in detailed analysis, i.e. marker-assisted selection (MAS) or map-based cloning.

Materials and methods

Selection of experimental materials by continuous backcrossing

The sequence developed to produce the experimental material is shown in Fig. 1. The F_1 plants, derived from a cross between 'Nipponbare' and 'Kasalath', were backcrossed with 'Nipponbare' as the male parent. By self-pollination of the BC_1F_1 plants, BC_1F_2 progeny were produced. The genotypes of each individual were surveyed by RFLP analysis as described below, and plants whose target QTL (*Hd-1*, *Hd-2* or *Hd-3*) genotypes were homozygous for 'Kasalath' alleles were crossed with 'Nipponbare' again. From the BC_2F_1 generation, selections and backcrosses were carried out to obtain suitable BC_3F_1 plants for analysis. These BC_3F_1 plants were regarded as F_1 plants for fine mapping of target QTLs because the putative QTL regions were heterozygous while almost all other regions were homozygous for 'Nipponbare' alleles. However, we could not obtain a suitable plant for *Hd-3* mapping in the series of $BC3F_1$ plants, so we selected an ideal individual (BC_3F_2-18-114) from the self-pollinated progeny of one BC_3F_1 plant. In the processes of developing each BC_1F_1, BC_1F_2 and BC_2F_1 plant, we used about 50 RFLP markers uniformly distributed on the 12 rice chromosomes, which were selected from the collection of genomic and cDNA clones reported by Kurata et al. (1994), and surveyed the genotype of each individual. The BC_3F_1 plants derived from selected BC_2F_1 plants were surveyed again for 128 RFLP markers selected from the same collection, and a graphical illustration of the genotype of each plant was drawn based on the concept proposed by Young and Tanksley (1989).

Field experiments

BC_3F_2 or BC_3F_3 populations were produced by self-pollination of BC_3F_1 or BC_3F_2 plants (Fig. 1). For convenience, these populations were called 94F2-5 ($n = 145$) for *Hd-1*, $94F_2$–13 (n = 94) for *Hd-2* and $95F_2$-18 ($n = 93$) for *Hd-3*, respectively. In 1994 (1995 for $95F_2$-18), these populations were cultivated in an experimental paddy field at the National Institute of Agrobiological Resources, Tsukuba, Ibaraki, Japan. At the tillering stage, 8 g of fresh leaves of each individual was collected for DNA extraction. Days to heading (defined as duration from sowing to emergence of the first panicle) was scored, and self-pollinated seeds of these BC_3F_2 or BC_3F_3 plants were collected. In 1995 (1996 for $95F_2$-18), the 50 progeny, of each F_2 plant were cultivated in a field. The genotype of each F_2 plant for their target QTLs was determined based on the status of segregation of days to heading in their progeny lines. In both years, cultivation was carried out in the natural season and according to standard practice.

Southern hybridization analysis

Total DNA from fresh leaf tissue was extracted by the CTAB method (Murray and Thompson 1980). Procedures for Southern hybridization analysis followed those of Kurata et al. (1994). For each sample, 2.4 micrograms of total DNA was digested with each of the following eight restriction enzymes, *Bam*HI, *Bgl*II,

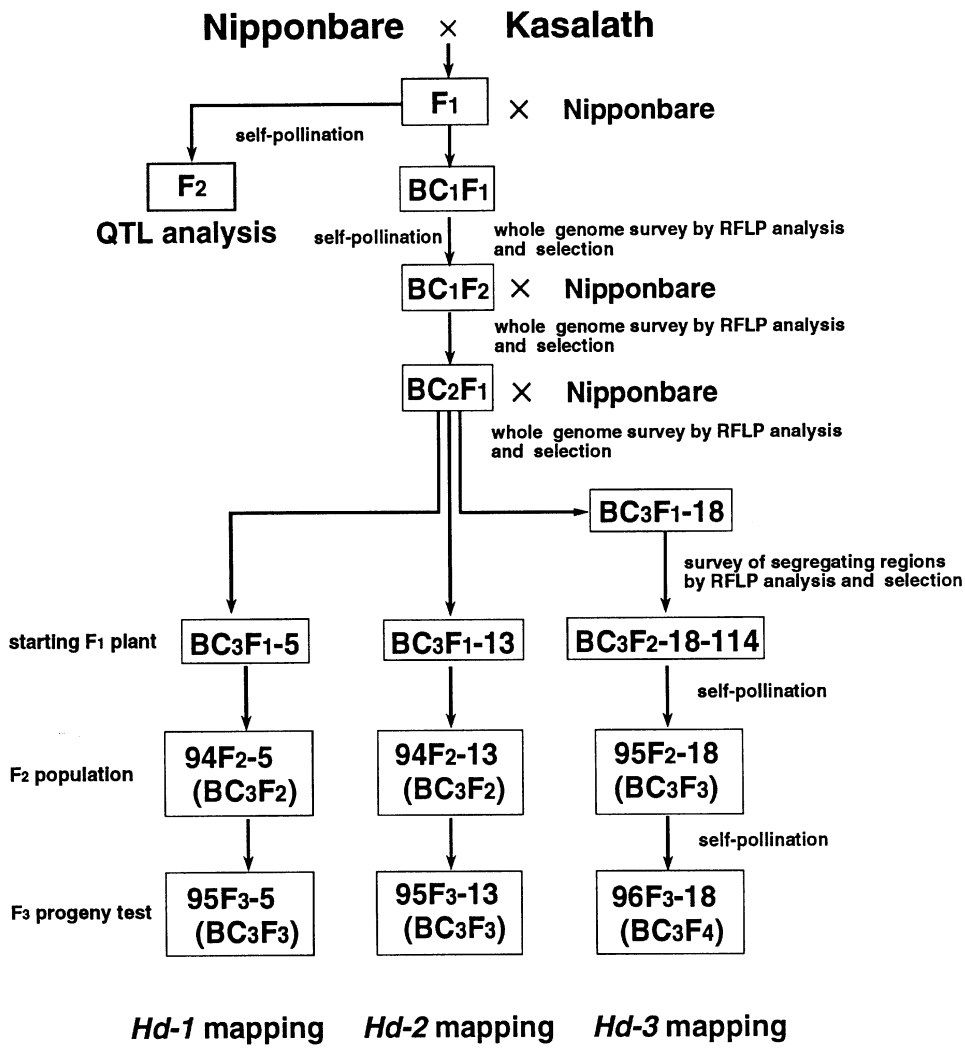

Fig. 1 Plant material used for fine mapping of QTLs controlling heading date in rice. Populations used in this study were derived from a cross between 'Nipponbare' (*japonica* variety) and 'Kasalath' (*indica* variety). Primary QTL analysis for heading date was carried out using an F_2 population (Yano et al. 1997)

*Eco*RV, *Hin*dIII, *Apa*I, *Dra*I, *Eco*RI and *Kpn*I, followed by electrophoresis in 0.6% agarose gel. DNAs were blotted onto a positively charged nylon membrane (Boehringer Mannheim) by capillary transfer mediated by 0.4 *N* NaOH. These blotted filters were washed with 2 × SSC, dried and baked at 120°C for 20 min. DNA clones for Southern hybridization were selected from the existing RFLP linkage map (Kurata et al. 1994), and insert DNAs were amplified by polymerase chain reaction (PCR) using M13 primers. DNA labeling, hybridization and detection of chemiluminescence on X-ray film were carried out using an ECL direct-labeling and detection system (Amersham).

Linkage mapping of putative QTLs

RFLP linkage maps in the QTL regions were constructed as follows: 53 RFLP markers were selected for $94F_2$-5 (population for analysis of *Hd-1*), 29 for $94F_2$-13 (population for analysis of *Hd-2*) and 23 for $95F_2$-18 (population for analysis of *Hd-3*). They were distributed on heterozygous regions of the selected BC_3F_1 or BC_3F_2 plants. Linkage analyses were performed by the MAPMAKER/EXP 3.0 program (Lander et al. 1987) using genotype data from both RFLP markers and target QTLs.

Results

Selection of experimental material

The genotypes of BC_3F_1 plants (BC_3F_1-5) selected for analysis of *Hd-1* were heterozygous for the *Hd-1* and *Hd-3* regions and homozygous for other QTL regions controlling heading date (Fig. 2A). According to Yano et al. (1997), the genetic effect of *Hd-3* is relatively much smaller than that of *Hd-1* (*Hd-1* explained about 66% of total phenotypic variation, while *Hd-3* was only detected in the secondary scanning analysis). Therefore, we concluded that while BC_3F_1-5 was not completely ideal experimental material, it could be used for the fine mapping of *Hd-1*. BC_3F_1-13, which was selected for analysis of *Hd-2*, has one heterozygous region (*Hd-2* region) controlling heading date (Fig. 2B). BC_3F_2-18-114, which was selected for analysis of *Hd-3*, has one heterozygous region (*Hd-3* region) controlling heading

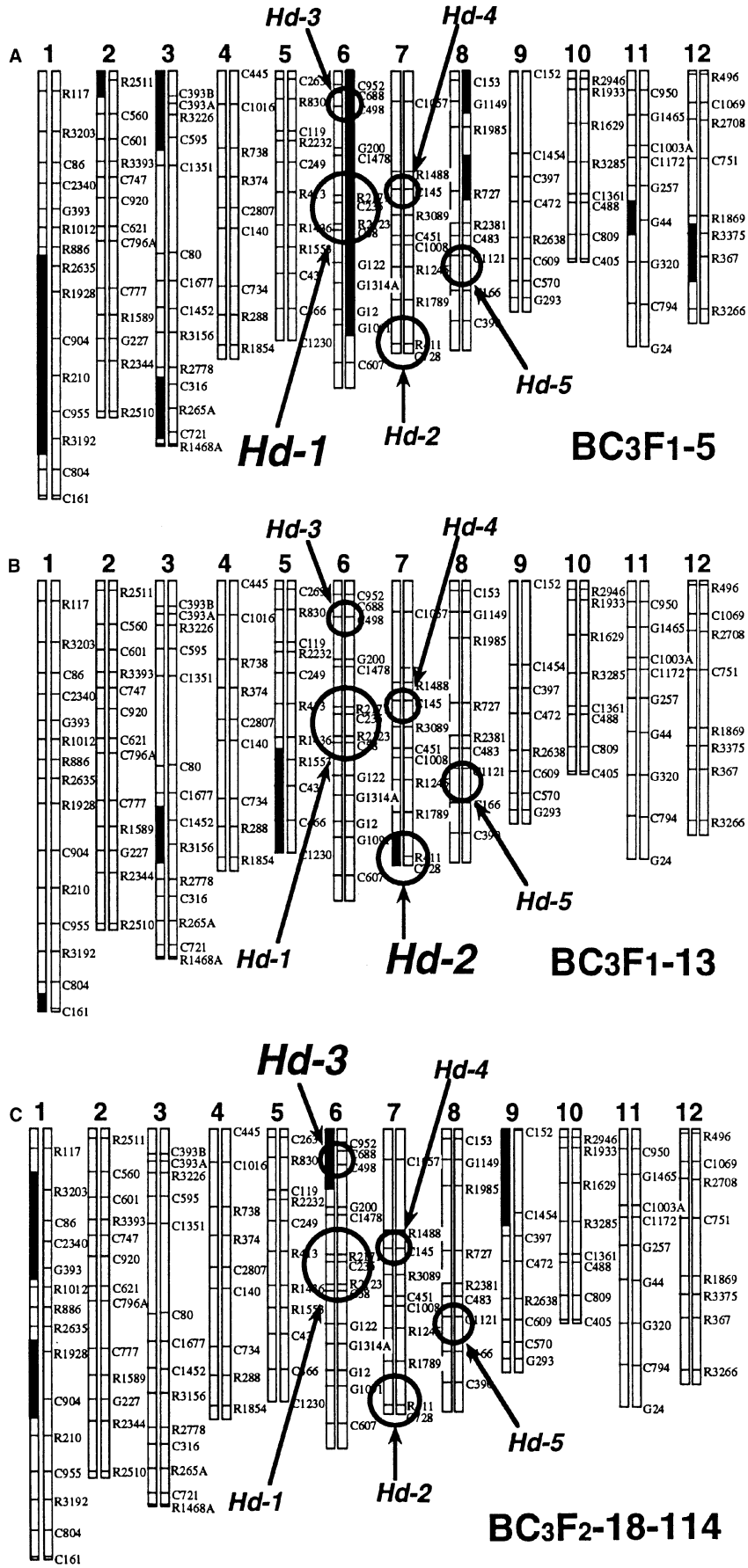

Fig. 2A–C Graphical genotypes of three plants, BC_3F_1-5 (**A**), BC_3F_1-13 (**B**) and BC_3F_2-18-114 (**C**). *Black* and *white* regions represent segments of the 12 chromosomes (*1–12*) derived from 'Kasalath' and 'Nipponbare', respectively. The *circles* indicate QTLs detected in the QTL analysis using the F_2 population (Yano et al. 1997), and the *size* of each *circle* indicates the relative magnitude of their genetic effect

date; the other QTL regions are homozygous for 'Nipponbare' alleles (Fig. 2C).

Mapping of *Hd-1*

The frequency distribution of days to heading in the population $94F_2$-5 ($n = 145$) is bimodal, between 95 days and 126 days, with 103 days as the boundary (Fig. 3A). The segregation of early and late types seemed to be in accordance with the expected Mendelian ratio (1 : 3). The QTLs that segregated in this population were *Hd-1* and *Hd-3* based on the graphical genotype (Fig. 2A). However, given the difference in magnitude of their genetic effects, this large variation was probably caused mainly by the segregation of *Hd-1*.

In BC_3F_3 progeny testing, three different phenotypes of heading – fixed lines of late heading, segregating lines of both types and fixed lines of early heading – were clearly visible. Therefore, it was easy to assess the genotype at *Hd-1* of each BC_3F_2 plant. The fixed lines of late heading were considered to be homozygous for 'Nipponbare' alleles, segregating lines as heterozygous plants and fixed lines of early heading as homozygous for 'Kasalath' alleles. The numbers of plants in these three groups were 33, 77 and 35, respectively, which fitted the Mendelian ratio for single-gene segregation ($\chi^2 = 0.61$). By means of linkage analysis using genotype data of both *Hd-1* and RFLP markers, we located *Hd-1* between RFLP markers R1679 and P130 and determined that it co-segregated with C235 (Fig. 4A).

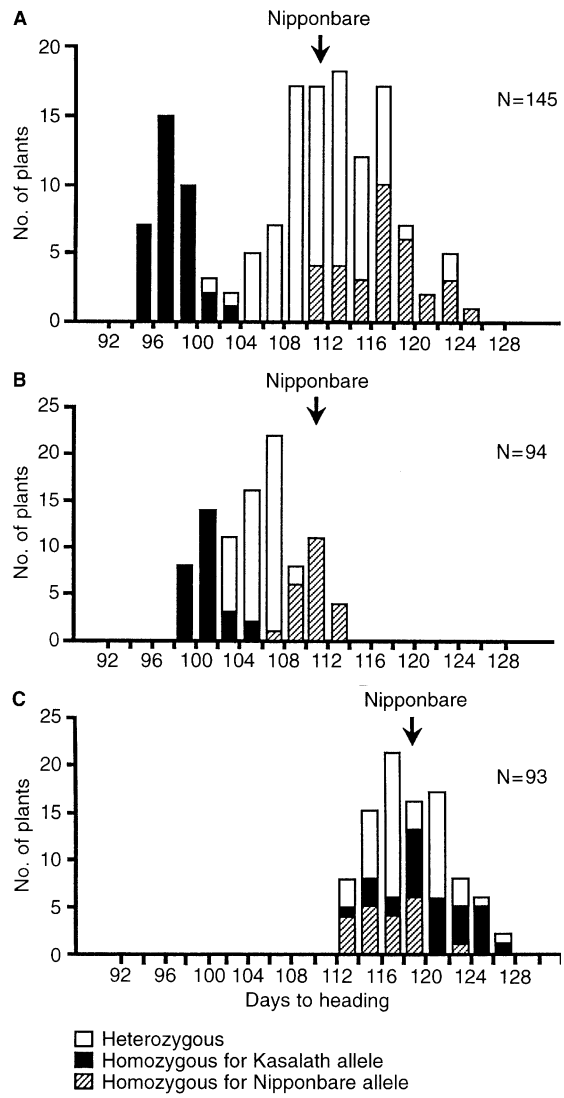

Fig. 3A–C Frequency distributions of days to heading in selfed progeny derived from selected backcrossed plants: $94F_2$-5 (**A**) is derived from BC_3F_1-5 for analysis of *Hd-1*, $94F_2$-13 (**B**) is derived from BC_3F_1-13 for analysis of *Hd-2*, $95F_2$-18 (**C**) is derived from BC_3F_2-18-114 for analysis of *Hd-3*. Three classified genotypes (homozygous for the 'Kasalath' allele, heterozygous and homozygous for 'Nipponbare') assessed by F_3 progeny tests are as indicated

Mapping of *Hd-2*

A large variation in days to heading was observed in the population of $94F_2$-13 ($n = 94$). This variation, which we attributed to the segregation of *Hd-2*, was continuous, resulting in a normal distribution ranging from 99 days to 113 days (Fig. 3B).

In the progeny testing, we found three different types of heading – late-fixed (homozygous for 'Nipponbare' at *Hd-2*), segregated (heterozygous) and early-fixed (homozygous for 'Kasalath'). The numbers of lines showing each phenotype were 22, 45 and 27, respectively. This pattern also fit the Mendelian ratio of single-gene segregation ($\chi^2 = 0.70$). By means of linkage analysis, we determined that *Hd-2* co-segregated with four RFLP markers, S2267, R411, S1979 and C728 on chromosome 7 (Fig. 4B).

Mapping of *Hd-3*

In the population $95F_2$-18 ($n = 93$), continuous and normal distribution of days to heading was observed (Fig. 3C). The range was 113–127 days and was shifted slightly towards late heading compared with 'Nipponbare'. Judging from the graphical genotype in Fig. 2C and the direction of shifting, which is, reverse of those of *Hd-1* and *Hd-2* (Yano et al. 1997), most of this variation was due to allelic differences at the *Hd-3* locus.

Progeny testing was performed, and the 93 progeny lines were classified into three groups: 20 lines for early heading (homozygous for 'Nipponbare' at *Hd-3*), 44 heterozygous, and 29 for late heading (homozygous for 'Kasalath'), respectively ($\chi^2 = 2.01$). On the basis of the genotype data for the *Hd-3* and RFLP markers, *Hd-3*

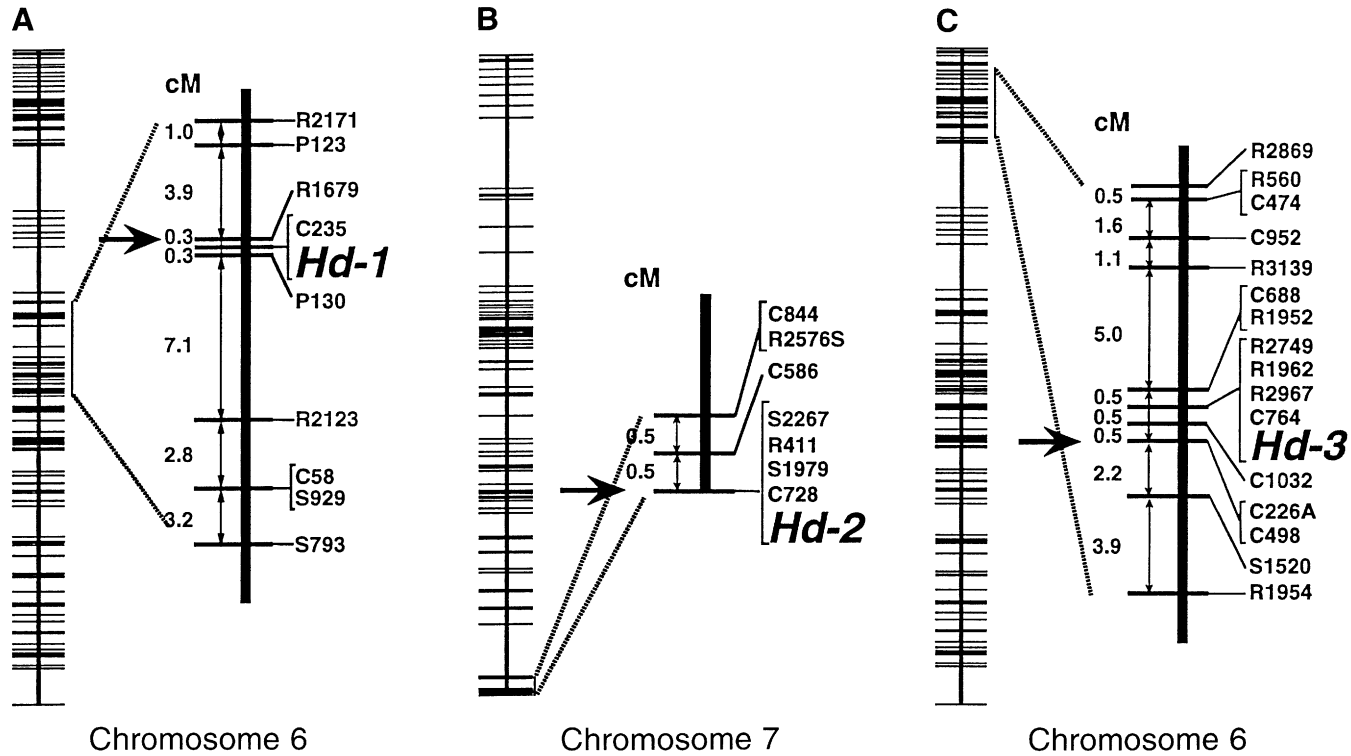

Fig. 4A–C Linkage maps of regions of chromosomes 6 and 7 showing the locations of three QTLs *Hd-1* (**A**), *Hd-2* (**B**) and *Hd-3* (**C**) controlling heading date. In each map, the *left vertical bar* indicates an RFLP linkage map constructed from the F_2 population of 'Nipponbare' and 'Kasalath' (Kurata et al. 1994). The *right vertical bar* represents the linkage map constructed in this study. The map distances (cM) were calculated by the Kosambi function and are shown on the *left side* of each map, while the names of markers and QTLs are shown on the *right side*. Nearest marker loci, which were estimated by MAPMAKER/QTL from analysis of the F_2 population (Yano et al. 1997), are indicated by arrows

was located between RFLP markers R1952 and C1032 and co-segregated with R2749, R1962, R2967 and C764 on chromosome 6 (Fig. 4C).

Discussion

Fine mapping of QTLs as Mendelian factors

Dorweiler et al. (1993) and Doebley et al. (1995) reported the linkage mapping of QTLs controlling ear shape, which is a key character in the differentiation of maize and teosinte. Alpert and Tanksley (1996) made a high-resolution genetic dissection of QTLs related to fruit weight in tomato for map-based cloning of the gene responsible. These studies clearly indicated that some major QTLs could be treated as single Mendelian factors in linkage mapping.

In this study, we precisely located as single Mendelian factors on the RFLP linkage map, three, *Hd-1*, *Hd-2* and *Hd-3*, of five QTLs controlling heading date in F_2 populations between 'Nipponbare' and 'Kasalath' that had been detected in a previous study (Yano et al. 1997). *Hd-1* was mapped on the centromeric region of chromosome 6; *Hd-2* was mapped on the distal end of chromosome 7; *Hd-3* was mapped on the distal region of chromosome 6. Though *Hd-1* and *Hd-2* were detected by a first scanning analysis in MAPMAKER/QTL, *Hd-3* was only detected by a multiple QTL model analysis. Additionally, the estimated additive effect of the 'Kasalath' allele at this QTL was only about 2 days in the F_2 population (Yano et al. 1997). This result clearly indicates that even a QTL with small allelic difference can be dealt with as a single Mendelian locus. Although only a case study, this finding provides a reasonable answer to the first question raised earlier; that is, whether a QTL corresponds to one locus or to a chromosomal region with a cluster of genes, each of which has relatively small genetic effect. It means that putative QTLs, including minor ones detected only by statistical analysis, have the same genetic basis and are amenable to the same genetic analysis as that used for known major genes.

Construction of QTL-NILs as analytical material

We consider that the successful fine mapping of QTLs was due to the use of suitable backcrossed progeny that

had been selected carefully by RFLP markers. Genetic analysis using NILs is a powerful method to estimate the chromosomal location and gene action of QTLs. Law (1966) clearly demonstrated that inter-varietal chromosomal substitution lines are useful for the mapping of QTLs in wheat. The recent development of molecular markers has allowed us to make just such substitutions of chromosome or chromosomal segments even in diploid species. Yamamoto et al. (1997), using continuously backcrossed progeny and RFLP analysis, detected the locus controlling spreading stub in rice, even though the phenotypes were too variable to allow categorization of the genotypes in the F_2 population. This strategy can be applied to the analysis of quantitative traits; that means, constructing NILs with target QTLs (QTL-NILs) based on the results of primary QTL analysis. After the advent of molecular markers to facilitated whole genome surveys, four studies (Dorweiler et al. 1993; Doebley et al. 1995; Alpert and Tanksley 1996; Tanksley et al. 1996) were carried out based on this concept. However, there have not been any examples in rice. Our study has demonstrated that QTL-NILs for rice heading date can be constructed rapidly using a whole genome survey with RFLP markers and that effective linkage mapping has been accomplished.

Reliability of QTL mapping and allelism with respect to known major genes

The five putative QTLs, *Hd-1* to *Hd-5*, detected earlier by QTL analysis based on a high-density linkage map comprised 857 DNA markers in which the average genetic distance between adjacent markers was 1.8 cM (Yano et al. 1997). These putative positions were almost the same as those mapped in this study (Fig. 4). We had previously tried QTL analyses in each backcrossed population ($94F_2$-5, $94F_2$-13 or $95F_2$-18) and had confirmed that the putative QTLs were located close to their previously reported positions (data not shown). These results clearly indicate that QTL analysis using a high-density RFLP linkage map provides reliable information for mapping QTLs.

The relationships between many putative QTLs and known major genes are uncertain. In maize, Beavis et al. (1991) hypothesized the possibility of coincidence between major genes determined by classical genetic analyses and QTLs estimated using molecular markers. Based on this hypothesis, Huang et al. (1996) verified some associations between QTLs for plant height and some dwarfing genes in rice. Yano et al. (1997) also indicated that some of the QTLs detected for heading date in rice were coincident with known major genes based on a comparison of their map locations. However, it is difficult to clarify the relationship between known major genes and QTLs because the experimental material or conditions used in each type of study were different. Allelism tests using NILs with the same genetic background or high-resolution fine mapping using the same DNA markers will make it possible to clarify this relationship. The closely linked RFLP markers, for example C235, C728 and R2967, will help to clarify the relationships between *Hd-1*, *Hd-2* or *Hd-3* and other candidate QTLs or major genes. However, the characterization of *Hd-1*, *Hd-2* and *Hd-3* still remains to be carried out. Yano et al. (1997) indicated that *Hd-1* and *Hd-3* might correspond to the photoperiod-sensitivity genes, *Se-1* and *En-Se-1*. In order to clarify the relationship between these photoperiod-sensitivity genes and QTLs, we need to investigate whether or not the QTLs involve an element of photoperiod sensitivity. The development of NILs for these QTLs in order to be able to analyze their functions is currently in progress (Yano and Sasaki 1997).

Application to marker-assisted selection (MAS) and map-based cloning

Mapping QTLs as single Mendelian factors will have a strong impact on breeding programs using MAS. Since a QTL can be a single locus, as demonstrated here, it will be possible to remove positively deleterious alleles in the vicinity of target QTLs (linkage drag) by MAS. High-density linkage mapping can make this feasible. By developing MAS of mapped QTLs, we will be able to perform QTL pyramiding, i.e. combining some QTLs having different gene actions into one variety. QTL pyramiding is an attractive method for both breeding strategies and basic research on epistatic interaction.

Recently, chromosomal substitution lines (introgression lines) covering the whole genome have been developed in tomato (Eshed and Zamir 1995) and *Brassica* species (Howell et al. 1996). Also, the concept of "advanced backcross QTL analysis" (AB-QTL analysis) was proposed by Tanksley and Nelson (1996) and used in tomato by Tanksley et al. (1996). These devices make QTL analysis and backcross breeding more effective and will provide suitable experimental material for the fine mapping of QTLs. In rice, similar lines were reported as preliminary-near-isogenic lines (PILs) for QTL analysis of blast resistance (Wang et al. 1994) and as backcross inbred lines (BILs) for QTL analysis of the regeneration ability of seed callus (Taguchi-Shiobara et al. 1997).

We have identified the precise location of QTLs by ordinary linkage mapping, which has become a standard starting point for map-based cloning (Tanksley et al. 1995). In plants, several economically important genes have been isolated by map-based cloning, including a photoperiod-sensitivity gene (flowering gene) in *Arabidopsis* (Putterill et al. 1995). The cloning of genes identified as QTLs has not been reported yet, although several groups are working in this area.

Acknowledgments We are grateful to Dr. K. Hasegawa for his advice and encouragement. This work was supported by funds from the Ministry of Agriculture, Forestry and Fisheries of Japan (MAFF), the Japan Racing Association (JRA) and the Program for Promotion of Basic Research Activities for Innovative Biosciences (PROBRAIN).

References

Alpert KB, Tanksley SD (1996) High-resolution mapping and isolation of a yeast artificial chromosome contig containing *fw2.2*: a major fruit weight quantitative trait locus in tomato. Proc Natl Acad Sci USA 93: 15503–15507

Beavis WD, Grant D, Albertsen M, Fincher R (1991) Quantitative trait loci for plant height in four maize populations and their associations with qualitative genetic loci. Theor Appl Genet 83: 141–145

Doebley J, Stec A, Gustus C (1995) *Teosinte branched1* and the origin of maize: Evidence for epistasis and the evolution of dominance. Genetics 141: 333–346

Dorweiler J, Stec A, Kermicle J, Doebley J (1993) *Teosinte glume architecture 1*: a genetic locus controlling a key step in maize evolution. Science 262: 233–235

Eshed Y, Zamir D (1995) An introgression line population of *Lycopersicon pennellii* in the cultivated tomato enables the identification and fine mapping of yield associated QTL. Genetics 141: 1147–1162

Howell PM, Marshall DF, Lydiate DJ (1996) Towards developing intervarietal substitution lines in *Brassica napus* using marker-assisted selection. Genome 39: 348–358

Huang N, Courtois B, Khush GS, Lin H, Wang G, Wu P, Zheng K (1996) Association of quantitative trait loci for plant height with major dwarfing genes in rice. Heredity 77: 130–137

Kurata N, Nagamura Y, Yamamoto K, Harushima Y, Sue N, Wu J, Antonio BA, Shomura A, Shimizu T, Lin SY, Inoue T, Fukuda A, Shimano T, Kuboki Y, Toyama T, Miyamoto Y, Kirihara T, Hayasaka K, Miyao A, Monna L, Zhong HS, Tamura Y, Wang ZX, Momma T, Umehara Y, Yano M, Sasaki T, Minobe Y (1994) A 300 kilobase interval genetic map of rice including 883 expressed sequences. Nat Genet 8: 365–372

Lander ES, Green P, Abrahamson J, Barlow A, Daley MJ, Lincoln SE, Newburg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1: 174–181

Law CN (1966) The location of genetic factors affecting a quantitative character in wheat. Genetics 53: 487–498

Li Z, Pinson SRM, Stansel JW, Park WD (1995) Identification of quantitative trait loci (QTLs) for heading date and plant height in cultivated rice (*Oryza sativa* L). Theor Appl Genet 91: 374–381

Lin HX, Qian HR, Zhuang JY, Lu J, Xiong ZM, Min SK, Huang N, Zheng KL (1996) RFLP mapping of major genes and minor genes for heading date in rice. Rice Genet Newsl 12: 253–255

Lu C, Shen L, Tan Z, Xu Y, He P, Chen Y, Zhu L (1997) Comparative mapping of QTLs for agronomic traits of rice across environments using a doubled-haploid population. Theor Appl Genet 94: 145–150

Mackill DJ, Salam MA, Wang ZY, Tanksley SD (1993) A major photoperiod-sensitivity gene tagged with RFLP and isozyme markers in rice. Theor Appl Genet 85: 536–540

McCouch SR, Doerge RW (1995) QTL mapping in rice. Trends Genet 11: 482–487

Murray MG, Thompson WF (1980) Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res 8: 4321–4325

Oosumi T, Miyazaki A, Uchimiya H, Kikuchi F, Yokoo M (1989) Analysis of glucose phosphate isomerase in near-isogenic lines and cultivars of rice (*Oryza sativa* L.) Bot Mag Tokyo 102: 283–289

Paterson AH, Lander ES, Hewitt JD, Peterson S, Lincoln SE, Tanksley SD (1988) Resolution of quantitative traits into Mendelian factors by using a complete map of restriction fragment length polymorphisms. Nature 335: 721–726

Paterson AH, Deverna JW, Lanini B, Tanksley SD (1990) Fine mapping of quantitative trait loci using selected overlapping recombinant chromosomes in an interspecies cross of tomato. Genetics 124: 735–742

Putterill J, Robson F, Lee K, Simon R, Coupland G (1995) The *CONSTANS* gene of *Arabidopsis* promotes flowering and encodes a protein showing similarities to zinc finger transcription factors. Cell 80: 847–857

Sano Y (1992) Genetic comparisons of chromosome 6 between wild and cultivated rice. Jpn J Breed 42: 561–572

Sato S, Sakamoto I, Shirakawa K, Nakasone S (1988) Chromosomal location of an earliness gene *Ef1* of rice, *Oryza sativa* L. Jpn J Breed 38: 385–396

Taguchi-Shiobara F, Lin SY, Tanno K, Komatsuda T, Yano M, Sasaki T, Oka S (1997) Mapping quantitative trait loci associated with regeneration ability of seed callus in rice, *Oryza sativa* L. Theor Appl Genet 95: 828–833

Tanksley SD (1993) Mapping polygenes. Annu Rev Genet 27: 205–233

Tanksley SD, Nelson JC (1996) Advanced backcross QTL analysis: a method for the simultaneous discovery and transfer of valuable QTLs from unadapted germplasm into elite breeding lines. Theor Appl Genet 92: 191–203

Tanksley SD, Ganal MW, Martin GB (1995) Chromosomal landing: a paradigm for map-based gene cloning in plants with large genomes. Trends Genet 11: 63–68

Tanksley SD, Grandillo S, Fulton TM, Zamir D, Eshed Y, Petiard V, Lopez J, Beck-Bunn T (1996) Advanced backcross QTL analysis in a cross between an elite processing line of tomato and its wild relative *L. pimpinellifolium*. Theor Appl Genet 92: 213–224

Wang GL, Mackill DJ, Bonman JM, McCouch SR, Champoux MC, Nelson RJ (1994) RFLP mapping of genes conferring complete and partial resistance to blast in a durably resistant rice cultivar. Genetics 136: 1421–1434

Xiao J, Li J, Yuan L, Tanksley SD (1995) Dominance is the major genetic basis of heterosis in rice as revealed by QTL analysis using molecular markers. Genetics 140: 745–754

Xiao J, Li J, Yuan L, Tanksley SD (1996) Identification of QTLs affecting traits of agronomic importance in a recombinant inbred population derived from a subspecific rice cross. Theor Appl Genet 92: 230–244

Yamamoto T, Sasaki T, Yano M (1997) Genetic analysis of spreading stub using *indica/japonica* backcrossed progenies in rice. Breed Sci 47: 141–144

Yano M, Sasaki T (1997) Genetic and molecular dissection of quantitative traits in rice. Plant Mol Biol 35: 145–153

Yano M, Harushima Y, Nagamura Y, Kurata N, Minobe Y, Sasaki T (1997) Identification of quantitative trait loci controlling heading date of rice using a high-density linkage map. Theor Appl Genet 95: 1025–1032

Yokoo M, Okuno K (1993) Genetic analysis of earliness mutations induced in the rice cultivar 'Norin' 8. Jpn J Breed 43: 1–11

Yokoo M, Kikuchi F, Nakane A, Fujimaki H (1980) Genetic analysis of heading date by aid of close linkage with blast resistance in rice. Bull Nat Inst Agric Sci Ser D 31: 95–126

Young ND, Tanksley SD (1989) Restriction fragment length polymorphism maps and the concept of graphical genotypes. Theor Appl Genet 77: 95–101