Two extremely late heading mutants were induced by ion beam irradiation in rice cultivar ‘Taichung 65’: KGM26 and KGM27. The F$_2$ populations from the cross between the two mutants and Taichung 65 showed clear 3 early: 1 late segregation, suggesting control of late heading by a recessive gene. The genes identified in KGM26 and KGM27 were respectively designated as $FLT1$ and $FLT2$. The two genes were mapped using the crosses between the two mutants and an Indica cultivar ‘Kasalath’. $FLT1$ was located on the distal end of the short arm of chromosome 8. $FLT2$ was located around the centromere of chromosome 9. $FLT1$ might share the same locus as $EHD3$ because their chromosomal location is overlapping. $FLT2$ is inferred to be a new gene because no gene with a comparable effect to that of this gene was mapped near the centromere of chromosome 9. In crosses with Kasalath, homozygotes of late heading mutant genes showed a large variation of days to heading, suggesting that other genes affected late heading mutant genes.

Key Words: late flowering, ion beam, mutation, linkage analysis, days to heading.

Materials and Methods

Plant material

Rice is classified into two varietal groups: Indica and Japonica. The Japonica rice cultivar ‘Taichung 65’ (T65) was used for ion beam irradiation because this cultivar has can induce various new alleles that might not be been present in nature. Detecting new loci controlling heading time and series of alleles originated from natural variation and those induced by artificial mutation contribute to the genetic and molecular mechanism controlling heading time. Knowledge of all those mechanisms is expected to facilitate efficient rice breeding with adequate heading time.

With the aid of Japan Atomic Energy Agency (JAEA) and the Inter-University Program for the Joint Use of JAEA Facilities, we started producing rice mutants with ion beams in 1999. We have obtained several extremely late heading mutants. This report describes the genetic analysis and linkage analysis of late heading-time genes in two mutant lines: KGM26 and KGM27 (Table 1).

Table 1. Days to heading (DH) of parental lines (ca. 20 plants × two replications) planted in 2010

| Parental line | Description                  | Days to heading (DH) |
|---------------|------------------------------|----------------------|
| T65           | Original cultivar            | 88.3                 |
| KGM26         | late-heading mutant line     | 148.8                |
| KGM27         | late-heading mutant line     | 143.7                |
| Kasalath      | an Indica cultivar used for molecular mapping of late heading time genes | 81.4                 |
often been used for studies of mutagenesis (Satoh et al. 2010, Suzuki et al. 2008) and heading time (Itoh et al. 2001, Khun et al. 2004, Sano 1992, Sato et al. 1992, Tsai 1976, 1986, 1991). Moreover, it has been used as genetic background of introgression lines and chromosomal segment substitution lines (CSSL) (Yasu et al. 2010, Yoshimura et al. 2010).

Hulled dry seeds of T65 were placed on 6-cm-diameter petri dishes, with the embryos facing the irradiation source, according to procedures described by Yamaguchi et al. (2009). Samples were irradiated with 220 MeV carbon ions at a dose of 25 Gy or 30 Gy, generated by an AVF-cyclotron (Takasaki Ion Accelerators for Advanced Radiation Application (TIARA) at JAERA, Takasaki, Japan). Plants heading later than T65 by about two months were identified in two M2 lines. M3 lines derived from selfing of the respective M2 plants were fixed for late heading. The progenies of the M3 lines were named KGM26 and KGM27: KGM26 were irradiated with 25 Gy; KGM26 were irradiated with 30 Gy.

An Indica cultivar, ‘Kasalath’, was used for mapping of the late heading-time genes because much polymorphism has been observed often between Kasalath and T65 (Ichitani et al. 2011).

Genetic analysis
KGM26 and KGM27 were crossed with T65 to ascertain the number of genes involved in late heading. KGM26 and KGM27 were also crossed with Kasalath. The F1 plants from the cross with Kasalath were selfed to produce F2 populations. They were also backcrossed with respective mutant lines to produce BC1F1 populations. The F2 plants from the cross between T65 and Kasalath were also planted as a ‘reference’ population. Parental lines (ca. 20 plants × two replications) were planted along with F2 and BC1F1 populations.

Germinated seeds were sown in nursery beds in a greenhouse. About two weeks after sowing, seedlings were transferred out of the greenhouse. About 30 days after the sowing date, seedlings were planted to a paddy field in the experimental field of the Faculty of Agriculture, Kagoshima University, Kagoshima, Japan. The heading date was recorded for each plant when the first developing panicle emerged from the leaf sheath of the flag leaf. The heading date was converted into days to heading (DH). Records of the heading date were kept until Dec. 1 (DH = 184). Some plants not heading before Dec. 2 were recorded as DH = 200. The applied fertilizers were 4, 6, and 5 g/m2 respectively, for N, K2O, and P2O5. Plant spacing was 15 × 30 cm. Sowing and transplanting were done respectively on May 31 and June 24 in 2010.

DNA analysis
In each population from the cross with Kasalath, 96 plants were used for PCR-based DNA marker-based linkage analysis. DNA was extracted according to the experimental protocols of the Rice Genome Project (RGP) (http://rgp.dna.affrc.go.jp/E/rgp/protocols/index.html, written in Japanese) with some modifications. Briefly, each leaf tip, 5 cm long from a single plant, was put on a well in a 96-deep-well plate. Then 250 μl of extraction buffer (100 mM Tris–HCl (pH 8.0), 1 M KCl, and 10 mM EDTA) was added with a 5-mm-diameter stainless steel ball in a well. After being covered with a hard lid, a plate was shaken hard (ShakeMaster ver. 1.2; BioMedical Science Inc.) for a minute to grind the leaves. After centrifuging, a plate was incubated at 70°C for an hour. Then 50 μl of supernatant was recovered and 50 μl of 2-propanol was added. After centrifuging, the supernatant was discarded and the DNA pellet was rinsed with 100 μl of 70% ethanol. The DNA pellet was dried and dissolved in 200 μl of sterilized distilled water. The PCR mixture (5 μl) contained 1 μl of template DNA, 200 mM of each dNTP, 0.2 μM of primers, 0.25 units of Taq polymerase (AmpliTaq Gold; Applied BioSystems), and 1 μl × buffer containing MgCl2. The PCR products were analyzed using electrophoresis in 10% (29:1) polyacrylamide gel, followed by ethidium bromide staining. They were viewed under ultraviolet light irradiation. Linkage analysis was conducted using a computer program (MapDisto ver. 1.7; Lorieux 2007). Map distances were estimated using the Kosambi function (Kosambi 1944).

DNA markers
For mapping the late heading-time genes, we used 81 published SSR and insertion/deletion (indel) markers, most of which included primer information from McCouch et al. (2002), IRGSP (2005), Rice Genome Research Program (http://rgp.dna.affrc.go.jp/E/publicdata/caps/index.html) and Ichitani et al. (2011). Because the target regions of the genes of interest were narrower, no published DNA markers were present there. Alternatively, none was applicable to linkage analyses. Therefore, we developed new PCR-based DNA markers (Table 2). We used indel information released by Xu et al. (2012). Then the obtained information was confirmed by comparing the genome sequences of a Japonica cultivar, ‘Nipponbare’, in Oryzabase (Yamazaki et al. 2010, http://www.shigen.nig.ac.jp/rice/oryzabaseV4/blast/search) and two Indica cultivars, ‘93-11’ and ‘PA64’, in the Rice Information System (Zhao et al. 2004, http://rise2.genomics.org.cn/page/rice/index.jsp). Primers surrounding indels were designed using Primer 3 (Rozen and Skaletsky 2000).

Results
The F2 populations from the cross between KGM26 and T65 and from the cross between KGM27 and T65 both showed clear bimodal distributions of DH: the ratio of early plants: late plants fitted 3: 1, one-gene segregation (KGM26: χ2 = 0.355, P = 0.55. KGM27: χ2 = 0.004, P = 0.95) (Fig. 1). These results suggest that the late heading characteristic of the two mutants was controlled respectively by a recessive gene. The F1 plants from the cross between the mutants and the original cultivar T65 showed DH similar to those of...
Table 2. Primer sequences designed and used for mapping mutant late heading time genes

| Marker name | Kind of DNA marker | Primer sequences | Location on IRGSP pseudomolecule Build 5 |
|-------------|--------------------|------------------|------------------------------------------|
|             |                    |                  | Chromosome | From   | To     | Source         |
| RM22196     | SSR                | TCTGTTCCCTCCGGTAGTGTGC | 8          | 108352 | 108560 | IRGSP 2005     |
|             |                    | CCCATCCATTCATACTCTTCG  |            |        |        |                |
| KG8C8M1     | Del                | TAAATCTAACCCGACATTGGCTTAC  | 8          | 199879 | 199977 | This study     |
|             |                    | AAGGTTGAAGTAGAGCGAGGAGA   | 8          | 201331 | 201477 | McCouch et al. 2002 |
| RM22220     | SSR                | GTTGGAGAAGAGTGGTGAGTTGATTGCCAGTCGAGG  | 8          | 233347 | 233502 | IRGSP 2005     |
| KG8C8M2     | Del                | TTCTACTGATTGGCTGCTAGCTTC  | 8          | 289390 | 289509 | This study     |
| KG8C8M3     | Del                | AAAATATGGGATGAAGAGTACACGAATCGCCTTCGAGGAGAC   | 8          | 322086 | 322235 | This study     |
| KG8C8M4     | Del                | GCGACTCTAGTGCCAGTCACTCCAG  | 8          | 467282 | 467477 | IRGSP 2005     |
| KG8C8M5     | Del                | GACTACGAGACGAGTGATTTGAACCTTCTGCTGGGAGAATGGCGGAGGAGAC   | 8          | 522421 | 522619 | McCouch et al. 2002 |
| KG8C8M6     | Del                | GTGCAAGAATTAAGGAGAACCTTCTGCTGGGAGAATGGCGGAGGAGAC   | 8          | 2011264 | 2011454 | McCouch et al. 2002 |
| KG8C8M7     | Del                | CCCTGAGACCTCTAGTTGAGG    | 8          | 2513408 | 2513552 | IRGSP 2005     |
| KG8C8M8     | Del                | ACCTCATTTTTTACCAATTGCAGCAG  | 9          | 453743 | 455824 | This study     |
| KG8C8M9     | Del                | CTTCCTGACCAAGTCTACCGAG  | 9          | 6028192 | 6028300 | Momma et al. 2006 |
| KG8C8M10    | Del               | ATGTGCATCATATTTAACCCGAGTAG  | 9          | 9647258 | 964777 | Ichitani et al. 2011 |
| KG8C8M11    | Del               | GAGTCGTTCACTCAGTCTCGAG  | 9          | 145374 | 145583 | This study     |
| KG8C8M12    | Del               | ATGTTGCTATCACATTAAACCCGTAGAAG  | 9          | 6647258 | 664777 | Ichitani et al. 2011 |
| KG8C8M13    | Del               | ATGCTGATCTACGCTCGGAGAAG  | 9          | 6647258 | 664777 | Ichitani et al. 2011 |
| KG8C8M14    | Del               | ATGCTGATCTACGCTCGGAGAAG  | 9          | 6647258 | 664777 | Ichitani et al. 2011 |
| KG8C8M15    | Del               | ATGCTGATCTACGCTCGGAGAAG  | 9          | 6647258 | 664777 | Ichitani et al. 2011 |
| KG8C8M16    | Del               | ATGCTGATCTACGCTCGGAGAAG  | 9          | 6647258 | 664777 | Ichitani et al. 2011 |
| KG8C8M17    | Del               | ATGCTGATCTACGCTCGGAGAAG  | 9          | 6647258 | 664777 | Ichitani et al. 2011 |
| KG8C8M18    | Del               | ATGCTGATCTACGCTCGGAGAAG  | 9          | 6647258 | 664777 | Ichitani et al. 2011 |
| KG8C8M19    | Del               | ATGCTGATCTACGCTCGGAGAAG  | 9          | 6647258 | 664777 | Ichitani et al. 2011 |
| KG8C8M20    | Del               | ATGCTGATCTACGCTCGGAGAAG  | 9          | 6647258 | 664777 | Ichitani et al. 2011 |

* http://rgp.dna.affrc.go.jp/E/publicdata/caps/index.html
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T65, supporting this idea (data not shown). From these results and subsequent linkage analyses, it seems readily apparent that a different single gene controls late heading characteristics of the two mutants. Therefore, the genes identified using the analyses of KGM26 and KGM27 were respectively named *FLOWERING TIME 1* (FLT1) and *FLOWERING TIME 2* (FLT2), according to the gene nomenclature system for rice (McCouch and CGSNL 2008).

The F₂ populations from the cross between T65 and Kasalath showed a unimodal distribution of DH, most of which ranged between the parents (Fig. 2A). A few plants showed DH slightly later than T65. However, no transgressive-segregants as late as KGM26 and KGM27 appeared. These results indicate that the major genetic factors controlling the heading time of Kasalath was similar to those of T65.

The DH in the F₂ population from the cross ‘KGM26 × Kasalath’ were 71–200 (Fig. 2B). Some plants headed later than KGM26, and two plants, recorded as DH = 200, did not head before Dec. 2. This population was divisible into two groups: an early group consisting of 86 plants that headed earlier than the latest plant in the F₂ population from the cross ‘T65 × Kasalath’ (DH = 94), and a late group consisting of 20 plants that headed later. The ratio 86: 20 fitted 3: 1, one-gene segregation ($\chi^2 = 2.126, P = 0.14$). These results suggest that the late group plants were recessively homozygous for KGM26 allele at the FLT1 locus.

The DH in the F₂ population from the cross ‘KGM27 × Kasalath’ were also 71 to 200 (Fig. 2C). This population can also be divided into two groups: the early group includes 87 plants that headed earlier than the latest plant in the F₂ population from the cross ‘T65 × Kasalath’; the late group includes 32 plants that headed later. The ratio of 87: 32 fitted 3: 1 ($\chi^2 = 0.227, P = 0.63$). These results suggest that the
late group plants were recessively homozygous of KGM27 allele at the *FLT2* locus.

The BC$_1$F$_1$ population ‘[(KGM26 × Kasalath) × KGM26]’ showed bimodal distribution and many transgressive-segregants toward late heading (Fig. 3A). A breakpoint of distribution around DH of 100 divided the population into 49 early plants and 51 late plants, which fitted 1: 1, one-gene segregation ($\chi^2 = 0.400$, $P = 0.84$). These results suggest that the variation of DH was mainly attributable to the segregation of the gene at the *FLT1* locus: early plants can be regarded as heterozygotes whereas late plants can be regarded as homozygotes for the mutant late heading gene.

Then, six early plants showing DH shorter than 85 and six late plants showing DH longer than 114 were subjected to preliminary linkage analysis with 43 DNA markers scattered on the whole rice genome with the result that *FLT1* was linked closely with DNA marker RM1019 located on chromosome 8.

Then linkage analysis using the 96 plants and polymorphic DNA markers around RM1019 was performed. The six DNA markers, KGC8M1, RM1019, RM22220, KGC8M2, KGC8M3, and KGC8M4, mutually cosegregated. Fig. 3A shows a histogram of DH separated by the genotype of KGC8M2. Heterozygotes were highly skewed toward early heading, and homozygotes of KGM26 allele were highly skewed toward late heading. Table 3 shows DH and genotypes for a series of DNA markers and *FLT1* of non-recombinants and informative recombinants. In the three recombinants L3-7, L4-21, and L4-26, which are expected to be heterozygotes of *FLT1* because of their short DH, recombination events occurred between KGC8M4 and RM22236, and between RM22196 and KGC8M1. *FLT1* should be located near the loci at which genotypes of the recombinants were heterozygous. Therefore, *FLT1* is located between RM22196 and RM 22236. The other recombinant L2-6, which should be homozygous of KGM26 allele because of its long DH, supports this idea. In the F$_2$ population from the cross between KGM26 and Kasalath, genotyping with KGC8M2 coincided almost completely with variation in DH (Fig. 2B), supporting our inference that *FLT1* is linked closely with KGC8M2. Therefore, under the assumption that *FLT1* cosegregates with KGC8M2, the linkage relation between *FLT1* and 13 DNA markers was depicted in Fig. 4 using 96 BC$_1$F$_1$ plants. This gene is located between RM22196 and RM22236. It cosegregates with six markers: KGC8M1, RM1019, RM22220, KGC8M4, KGC8M3, and KGC8M2. The linkage around *FLT1* locus was compared with a restriction fragment length polymorphism (RFLP) marker-based high-density linkage map (Harushima et al. 1998) in which some RFLP markers have been sequenced. Based on the Nipponbare genome sequence, DNA markers

Table 3. Genotypes of representative recombinants and non-recombinants for the DNA marker loci linked with *FLT1* on chromosome 8 in the BC$_1$F$_1$ population [(KGM26 × Kasalath) × KGM26]

| Individual | Days to heading | *FLT1* | RM22196 | KGC8M1 | RM1019 | RM22220 | KGC8M2 | KGC8M3 | RM22236 | RM22239 | RM22252 | RM6863 | RM22355 | KGC0135 |
|------------|----------------|--------|---------|--------|--------|---------|--------|--------|---------|---------|---------|--------|---------|---------|
| L1-1       | 81             | H      | H       | H      | H      | H       | H      | H      | H       | H       | H       | H      | H       | H       |
| L1-15      | 80             | H      | H       | H      | H      | H       | H      | H      | H       | H       | H       | H      | H       | H       |
| L1-7       | 90             | H      | H       | H      | H      | H       | H      | H      | H       | H       | H       | H      | H       | H       |
| L4-21      | 83             | H      | J       | J      | J      | J       | J      | J      | J       | J       | J       | J      | J       | J       |
| L3-7       | 85             | H      | H       | H      | H      | H       | H      | J      | J       | J       | J       | J      | J       | J       |
| L4-26      | 89             | H      | H       | H      | H      | H       | H      | H      | J       | J       | J       | J      | J       | J       |
| L1-11      | 167            | J      | J       | J      | J      | J       | J      | J      | J       | J       | J       | J      | J       | J       |
| L1-18      | 146            | J      | J       | J      | J      | J       | J      | J      | J       | J       | J       | J      | J       | J       |
| L2-6       | 128            | J      | J       | J      | J      | J       | J      | J      | J       | J       | J       | J      | J       | J       |

*H and J respectively denote heterozygotes and homozygotes for KGM26 allele.*
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Located near each other on Nipponbare pseudomolecules are connected with dotted lines (Fig. 4): FLT1 is located on the distal end of the short arm of rice chromosome 8.

The BC1F1 population [(KGM27 × Kasalath) × KGM27] also shows a bimodal distribution, and many transgressive-segregants toward late heading (Fig. 3B). A breakpoint around DH of 97 divided the population into 55 early plants and 41 late plants, which fitted 1:1 (χ2 = 2.041, P = 0.15). These results suggest that the variation of DH was attributable mainly to the segregation of genes on the FLT2 locus.

Along with the mapping of FLT1, linkage analysis of FLT2 was performed using the same strategy. Preliminary analysis using six early plants showing DH shorter than 85 and six late plants showing DH longer than 120 with 70 DNA markers showed the linkage of FLT2 with RM23564 located on chromosome 9. Then linkage analysis using the 96 plants in the mapping population and polymorphic DNA markers was performed around RM23564. Five markers mutually cosegregated: RM23668, KGC9M6, KGC9M7, KGC9M8, and KGC9M9. Another set of five markers also mutually cosegregated: KGC9M1, RM23654, KGC9M4, KGC9M5, and KGC9M2. Table 4 shows DH and genotypes of a series of DNA markers and FLT2 of non-recombinants and informative recombinants. The recombinants L1-23, L1-26, L2-7, and L1-16 showed that FLT2 is located between KGC9M2 and KGC9M11. Fig. 3B shows a histogram of DH separated by the genotype of KGC9M7. Heterozygotes were highly skewed toward early heading. Homozygotes of KGM27 allele were highly skewed toward late heading. In the F2 population from the cross between KGM27 and Kasalath, genotypes of KGC9M7 coincided almost completely with variation in DH (Fig. 2C). These results support the inference that FLT2 is linked closely with KGC9M7. Therefore, under the assumption that FLT2 cosegregates with KGC9M7, the linkage relation among FLT2 and 18 DNA markers using 96 BC1F1 plants is depicted in Fig. 5: FLT2 is located between KGC9M2 and KGC9M11. It cosegregated with five markers: KGC9M6, KGC9M7, KGC9M8, KGC9M9, and RM23654. Comparison with the map presented by Harushima et al. (1998) shows that FLT2 is located around the centromere of rice chromosome 9.

Discussion

Two mutant late heading-time genes with a large effect were identified in this study. They were mapped on the rice genome: FLT1 is located on the 359 kb region on the distal end of the short arm chromosome 8 (Fig. 4, Table 3), and FLT2 is located on the 5,759 kb region around the centromere of chromosome 9 (Fig. 5, Table 4). Recombination events were, in general, highly suppressed around the centromere. In this study, two markers on chromosome 9, RM23668 and KGC9M9, were 4,169 kb distant from each other. However,

Table 4.

| Indiv. | Days to heading | FLT2 | Genotypes of the DNA marker loci linked with FLT2 on chromosome 9 in the BC1F1 population [(KGM27 × Kasalath) × KGM27] |
|-------|----------------|------|----------------------------------------------------------------------------------------------------------------|
| L1-14 | 77             | H    | KGC9M1, RM23654, KGC9M4, KGC9M5, KGC9M2, KGC9M3, KGC9M4, RM23654, KGC9M6, RM22339, RM22252 |
| L1-24 | 77             | H    | H H H H H H H H H H H H H H H H H H H H H H |
| L1-23 | 80             | H    | H H H H H H H H H H H H H H H H H H H H H H |
| L1-26 | 79             | H    | J J J J J J J J H H H H H H H H H H H H H H |
| L1-12 | 144            | J    | J J J J J J J J J J J J J J J J J J J J J J |
| L2-3  | 140            | J    | J J J J J J J J J J J J J J J J J J J J J J |
| L2-7  | 200            | J    | J J J J J J J J J J J J J J J J J H H H H H H H H H H H |
| L1-16 | 131            | J    | H H H H H H H J J J J J J J J J J J J J J J J |

a H and J respectively denote heterozygotes and homozygotes for KGM27 allele.
no recombinant was detected between them. Matsubara et al. (2011) reported that the causal gene of a late heading mutant from a Japonica cultivar 'Tohoku IL9' was Os08g0105000, encoding a nuclear protein that contains a putative transcriptional regulator with two plant homeodomain (PHD) finger motifs, mapped on the distal end of the short arm of chromosome 8. Matsubara et al. (2011) named this gene Ehd3 (Early heading date 3). The chromosomal region of FLT1 contains the location of Ehd3. Therefore, the possibility exists that FLT1 and Ehd3 are on the same locus. High-resolution mapping of FLT1 and sequencing Ehd3 gene on KGM26 and T65 must be done to ascertain these genes’ mutual relation.

As for FLT2, no gene with a comparable effect to that of this gene has been mapped near the centromere on chromosome 9. Recombination events were, in general, highly suppressed around the centromere, which suggests that it might be difficult to identify FLT2 using a map-based strategy. On the linkage map presented by Harushima et al. (1998), 21 markers were located between the map positions of 0.5 and 2.0. Among them, 12 markers were located on IRGSP Build5 Pseudomolecules of the Rice Genome. The map positions 0.5 and 2.0 respectively correspond to 880,493 (S13769) and 5,341,667 (G1047). No recombination occurred in the region in the [(KGM27 × Kasalath) × KGM27] population (Fig. 5). These facts suggest that the candidate region of FLT2 can be narrowed somewhat using a larger mapping population. However, the two physically distant markers, G95 (1,271,123 on IRGSP Build5 Pseudomolecules) and C1132 (4,414,047), were at the same map position of 0.8 in a study by Harushima et al. (1998) using 186 F2 plants from the cross between Nipponbare and Kasalath. Map-based cloning of this gene will be extremely difficult if FLT2 is located around the two markers.

The continuous variation in DH in the crosses with Kasalath suggests that some genes modify the effect of mutant late heading genes on DH. The F2 population from the cross between T65 and Kasalath showed slight variation in DH with a few late transgressive-segregants, suggesting that the major genetic factors controlling DH are similar in both cultivars. Regarding Sel (=HD1) locus, a major locus controlling photoperiod sensitivity, both Kasalath and T65 proved to carry a photoperiod insensitive allele (Ichitani et al. 1998, Inoue et al. 1998). However, Yano et al. (2001) reported that as many as 14 QTLs have been detected in the cross between Nipponbare and Kasalath. According to Okumoto et al. (1991, 1992), T65 and Nipponbare differ in the genotype of E2, E3, Sel, and ef1 loci. These facts suggest that genes on at least ten loci were segregating in the crosses between T65 and Kasalath. Therefore, the wide variation in DH of homozygotes of mutant genes in F2 and BC1F1 populations indicates that effects of the late heading mutant genes were affected strongly by these loci. Identification of the loci modifying the effect of the mutant genes will contribute to the understanding the genetic mechanism controlling the heading time in rice.

Many mutants and isogenic lines differing in heading time genes under T65 genetic background have been developed by Dr. Tsai (Tsai 1976, 1986, 1991), Dr. Sato and his colleagues (Khun et al. 2004, Sato et al. 1992), and Dr. Sano and his colleagues (Dung et al. 1998, Itoh et al. 2001, Sano 1990, 1992). We have selected KGM26, KGM27 and three other ion-beam induced extremely late heading mutants under T65 background (unpublished data). Allelism tests and pyramiding genes done by crossing these lines will improve our understanding of interactions among heading-time genes accurately under the same genetic background. Combining the isogenic lines by Tsai (1976) and Itoh et al. (2001), Uwakoto et al. (2008) revealed epistasis among the three major flowering time genes Sel (=Hd1) (Saito et al. 2011), Ef1 (=Ehd1) (Saito et al. 2009), and E1 (=Ghd7) (Saito et al. 2011). Adding our late heading mutants will contribute further to such studies.

Characterization of the mutant genes is urgently necessary. For the correct evaluation of basic vegetative growth and photoperiod sensitivity, mutants and the original cultivars should be grown under various daylength conditions at controlled temperatures. Temperatures are especially important for evaluating our late heading mutants: under natural conditions in fall, low temperatures prolong the days to

**Fig. 5.** Linkage map showing the location of FLT2 on rice chromosome 9: A, RFLP framework map of chromosome 9 modified from Harushima et al. (1998); B, Linkage map of FLT2 constructed from backcross population [(KGM27 × Kasalath) × KGM27] (n = 96). DNA markers that are located near each other on Nipponbare pseudomolecules are connected with dotted lines.
heading, causing overestimation of the photoperiod sensitivity and/or basic vegetative growth. We are therefore undertaking characterization of the mutant genes under controlled daylength and temperature conditions.

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