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High Potential of a Transposon mPing as a Marker System in japonica × japonica Cross in Rice

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Abstract
Although quantitative traits loci (QTL) analysis has been widely performed to isolate agronomically important genes, it has been difficult to obtain molecular markers between individuals with similar phenotypes (assortative mating). Recently, the miniature inverted-repeat transposable element mPing was shown to be active in the japonica strain Gimbozu EG4 where it had accumulated more than 1000 copies. In contrast, most other japonicas, including Nipponbare, have 50 or fewer mPing insertions in their genome. In this study we have exploited the polymorphism of mPing insertion sites to generate 150 PCR markers in a cross between the closely related japonicas, Nipponbare × Gimbozu (EG4). These new markers were distributed in genic regions of the whole genome and showed significantly higher polymorphism (150 of 183) than all other molecular markers tested including short sequence repeat markers (46 of 661). In addition, we performed QTL analysis with these markers using recombinant inbred lines derived from Nipponbare × Gimbozu EG4, and successfully mapped a locus involved in heading date on the short arm of chromosome 6. Moreover, we could easily map two novel loci involved in the culm length on the short arms of chromosomes 3 and 10.

Key words: Linkage mapping; Transposon; japonica; Oryza sativa L.; QTL analysis

1. Introduction

The development of DNA markers has made it possible to study the naturally occurring allelic variation controlling quantitative traits loci (QTL).1–2 QTL analysis based on high-resolution linkage maps is now routinely performed in various plant species and has resulted in the mapping loci of many genes involved in agronomically important traits.3–9 A high-resolution, high-density linkage map of rice has been constructed10–12 and used for QTL analyses that identified loci and genes involved in heading date13–16 and culm length.17 Like most other QTL analyses in rice, this was performed using crosses between the subspecies indica and japonica where it is relatively easy to obtain molecular markers and construct saturated linkage maps. However, the use of such distant cross combinations can disturb efficient identification of QTLs because of the prevalence of hybrid seed sterility and the simultaneous segregations of many other loci. As such, it is difficult to identify loci or alleles of minor effect. To do so usually requires the generation of additional genetic resources including near isogenic lines or
Transposable elements (TEs) have also been exploited in the development of molecular markers.\textsuperscript{28–33} In this regard, the most valuable TEs are those that are actively transposing and generating insertion site polymorphisms. Unfortunately, the rice genome is relatively stable with very few actively transposing elements. So far, only two TEs have been employed as markers among temperate \textit{japonica} varieties.\textsuperscript{27} Although TE insertion polymorphism was shown to be more frequent (42.3\%) than other DNA markers, the total number of applicable TE markers was only 52.\textsuperscript{27}

Here we report the successful generation of molecular markers using the newly characterized \textit{mPing} element. This element was independently discovered by three labs as the first active miniature inverted-repeat transposable element as well as the first active DNA transposon in rice.\textsuperscript{34–36} A subsequent study revealed that \textit{mPing} copy number was generally less than 50 in most cultivars, but that it had amplified to over 1000 copies in one strain, Gimbozu EG4 (EG4, hereafter). Thus, there are more than 1000 insertion site polymorphisms of \textit{mPing} when EG4 is compared with other cultivars including all characterized strains of the \textit{japonica} subspecies. Of these 1000 insertions, most of them (90\%) were into the single copy regions, and more than 70\% were located within 5 kb of transcribed DNA.\textsuperscript{37}

In this study, we have designed 150 sequence characterized amplified region (SCAR) markers based

\begin{table}[h]
\centering
\caption{Summary of QTL analysis among temperate \textit{japonica} × \textit{japonica} cross combinations}
\begin{tabular}{|l|l|l|l|l|l|}
\hline
Cross cultivars & Population structure & Markers used & No. of polymorphic markers/ no. of screening markers (polymorphism frequency, \%) & Total length, cM & Reference \\
\hline
Akihikari & DHLs & RFLP & 127/1252 (10.1) & No data & 23 \\
Koshihikari & & RAPD & 28/862 (3.0) & & \\
Chiyonishiki & RILs & SSR & 199/1663 (11.9) & 891.1 & 25 \\
Koshijiwase & & & & & \\
Hana-echizen & F2, F3 & SSR & 64/407 (15.7) & No data & 19 \\
Nigatawase & & & & & \\
Koshihikari & BILs & SSR & No data (11.8) & 956.1, 973.1 & 26 \\
Nipponbare & & SNP & No data (?) & & 50 \\
Sakihikari & RILs & SSR & No data (?) & No data & 27 \\
Nipponbare & & & & & \\
Moritawase & RILs & SSR & 130/1780 (7.3) & 1060 & 24 \\
Koshihikari & & & & & \\
Suweon365 & RILs & SSR & 121/621 (19.5) & 2227 & 27 \\
Chucheongbyeo & & AFLPs & 118/908 (12.9) & & 27 \\
& & MITEs & 52/71 (42.3) & & \\
Gimbozu & RILs & \textit{mPing}-SCAR & 150/183 (82.3) & 1771 & This study \\
Nipponbare & & SSR & 46/661 (7.0) & & \\
\hline
\end{tabular}
\end{table}
on the sequence information of mPing insertion sites in EG4. We will discuss the advantage of this novel marker system, which can be easily applicable for genetic analyses between closely related genomes.

2. Materials and methods

2.1 Plant materials and genomic DNA extraction

In 2005, a total of 190 F5 recombinant inbred lines (RILs) (12 plants per line) of EG4 × Nipponbare were grown in the paddy field of Kyoto University (35°01′N) under natural day length conditions. Seeds were sown on 17 May and transplanted on 24 June in an irrigated rice field. The heading date of those plants was also determined by calculating the average value of each line, monitoring for the appearance of the first panicle. Leaf material for DNA extraction was collected after monitoring heading date to minimize damage to F5 plants.

In 2007, a total of 96 F7 RILs (12 plants per line) were grown under the same conditions as described above. Seeds were sown on 1 May and transplanted on 24 May in an irrigated rice field. The culm length of those plants was measured to the top internodes. The heading date of those plants was also determined as described above. Genomic DNA of all plant materials was extracted by the cetyl tri-methylammonium bromide method.38

2.2 SSR markers

PCR for SSR markers was performed in 5 μL containing 0.5 μM 10× Ex-Taq buffer, 0.5 μM dNTPs (2 mM each), 0.25 μM DMSO, 0.02 μM Ex taq, 100 ng genomic DNA and 5 pmol of each primer. PCR conditions were as follows: 94°C for 10 min; 35 cycles of 94°C for 30 s, 50°C for 1 min and 72°C for 30 s; 72°C for 7 min.

Reaction products were loaded on 12% polyacrylamide gels, run at 500 v for 2 h, stained with ethidium bromide and gels were scanned with molecular imager FX (Nippon Bio-Rad Laboratories, Tokyo).

2.3 mPing-SCAR markers

Primers flanking mPing insertion sites in EG4 were designed based on sequence information from a previous study.37 PCR for mPing-SCAR markers was performed in 5 μL containing 0.5 μM 10× Ex-Taq buffer, 0.5 μM dNTP mix (2 mM each), 0.25 μM DMSO, 0.02 μM Ex-Taq, 100 ng genomic DNA and 8 pmol of each primer. The cycling parameters were as follows: 94°C for 3 min; 30 cycles of 95°C for 30 s, 58°C for 45 s and 72°C for 3 min; 72°C for 3 min.

Amplicons of mPing-SCAR markers were resolved in 1.0% agarose gels, stained with ethidium bromide and visualized under UV light.

2.4 Determining mPing excision frequency

mPing excision was assessed by PCR. Primers were designed flanking six mPing insertion sites that are shared by Nipponbare and EG4. Their sequences are: AC107315_U1: TGAATAAACATAGCCCATACG, AC107315_L1: AGGGTTTTTGGATACGAATG, AC083492_U1: GATGGTTGATGGTAGTTGAGAG, AC083492_L1: TGGCTGTAATAGTTTGGGGGTAG, AC121491_U1: GGTAACTGTAAGCCTAGTG, AC121491_L1: GAGAGCATTCCAAACGAATAAT, AP003542_U1: TTGGGTAGGGTT GTGGGGATT, AP003542_L1: GAGATGGGACTTTGAG GAGAGC, AP003634_U1: AAAAGAAAAACAGAAAAGATCG, AP003634_L1: CGCTCAACATAAACAAAAACC, BX000500_U1: AGTTGATACTAGTCTCTA, and BX000500_L1: CTTTCTTTCTCTCTCTCTTTTC. For EG4-specific insertion sites, 20 primer pairs of mPing-SCAR markers (MK1_1, MK1_10, MK1_12, MK1_13, MK1_22, MK1_23, MK1_28, MK1_29, MK1_30, MK1_32, MK2_1, MK2_2, MK2_4, MK2_5, MK2_8, MK2_23, MK3_2, MK3_9, MK3_13 and MK5_2) were chosen to analyze excision. PCR conditions were the same as described above.

2.5 Mapping of mPing-insertion sites

Chromosomal distribution of the mPing-SCAR markers and SSR markers were analyzed with 190 F5 RILs derived from a cross between EG4 and Nipponbare. Linkage analysis was performed with Mapmaker version 3.0.39

2.6 QTL analysis of heading date and culm length

Composite interval mapping,40 which combines interval mapping with multiple regression, was done using Windows QTL Cartographer 2.0 with forward and backward regression.41 The experiment-wise LOD threshold significance level was determined by computing 1000 permutations,42 as implemented by the QTL cartographer.

3. Results and discussion

3.1 Screening for SSR markers

SSR markers were first used for genotyping Nipponbare/EG4 RILs. However, only 46 of 661 markers tested (7.0%) were polymorphic. In addition, the distribution of these markers was uneven. Only a few markers were found polymorphic on chromosomes 3, 5, 6 and 9–12. In addition, there were long stretches of the genome without any markers on each of the 12 chromosomes of rice (see Fig. 1
Figure 1. The genetic map based on the mPing-SCAR markers and SSR markers. mPing-SCAR markers are shown in black whereas SSR markers are shown in red (see text for details).
for details). These results illustrate the difficulty of using conventional molecular markers for linkage analysis of *japonica × japonica* cross.

### 3.2 Designing mPing-SCAR markers

In a prior study it was determined that there were over 1000 insertion site polymorphisms of *mPing* between EG4 and Nipponbare.\(^{37}\) To exploit these polymorphisms in the development of molecular markers, we designed primer pairs based on genomic sequences flanking carefully selected *mPing* insertions (Fig. 2A). Insertion site polymorphisms were easily detected in both homozygous and in heterozygous genotypes, indicating that these primer sets could serve as co-dominant markers (Fig. 2B). The genotypes could be easily determined by 15-min electrophoresis in 1.0% agarose gels (Fig. 2B). Of 219 primer sets tested, 183 were successfully amplified, and 150 were found to be polymorphic between EG4 and Nipponbare.

### 3.3 Stability of mPing-SCAR markers

Transposition usually involves two events, element excision and element reinsertion elsewhere in the genome. In the previous finding, there are approximately 50 new *mPing* insertions per plant per generation in EG4.\(^{37}\) If *mPing* excises frequently, it has no value as a molecular marker. Thus, the excision frequency of six insertion sites was checked in 400 Nipponbare and 399 EG4 plants by PCR with flanking primers. Of all insertion sites tested, not a single empty site was detected (data not shown). In addition, 20 EG4-specific insertion sites in 96 EG4 plants were also tested for excision events by PCR using flanking primers. Only one plant showed a single excision at MK1_22 on chromosome 1 (see Materials and methods for details and Fig. 1). No empty sites were detected in any other plants (data not shown).

Based on the haploid copy number of EG4 (1163)\(^ {37}\) and the average number of new insertions (50)\(^ {37}\) per plant per generation, the probability of excision for any single copy of *mPing* should be 0.0215 (= 50/ (1163 × 2)). However, the observed excision frequency in the 96 EG4 plants was 80-fold lower than the expected value (only 0.00 026 (=1/(20 × 96 × 2))). How can there be so many new insertions without any apparent excision of *mPing*? This contradiction can be explained if *mPing* moves by the gap repair mechanism where double-strand breaks generated by *mPing* excisions are repaired by utilizing a copy of *mPing* from either the sister chromosome or from the homologous chromosome.\(^ {43,44}\) In total, *mPing* excisions are very rare events and do not disturb linkage analysis.

On the other hand, segregation distortion has always been observed in other linkage analysis. For example, in the F\(_2\) population of Nipponbare (*japonica*) × Kasalath (*indica*), 11 loci were detected to deviate from the expected 1:2:1 segregation.\(^ {45}\) Kwon et al.\(^ {31}\) also reported that 40–60% of the markers using insertion polymorphisms of TEs showed distorted segregation in the RILs of Milyang23 (*indica*) × Gihobyeo (*japonica*). In contrast, when 190 F\(_5\) RILs were surveyed with the 150 SCARs, the segregation ratio of genotypes (EG4:Nipponbare) was 1:1.01 (16 356:16 502), indicating that segregation was not significantly distorted. While this can be attributed to the close relationship between EG4 and Nipponbare, it also demonstrates the reliability of *mPing*-SCAR markers. However, it should be noted that the frequency of genotypes of the 150 *mPing*-SCAR marker-loci (EG4:Nipponbare =16 356:16 502) was slightly lower than that of the 46 SSR marker-loci (3943:3894). This may reflect *mPing* activity in the EG4 background, that is, some of the *mPing* elements may have excised.

### 3.4 Genetic mapping of mPing-SCAR and SSR markers

We constructed a genetic map (with Mapmaker version 3.0) that included 150 *mPing*-SCAR markers and 46 SSR markers in all 12 linkage groups (Fig. 1; Table 2 and see Supplementary Table S1). The total length of the genetic map was 1771 cM with an average genetic distance between markers of 9.0 cM. The number of markers per chromosome ranged from 9 on chromosome 9 to 31 on chromosome 1, and the length of genetic region per chromosome was from 92.9 cM (chromosome 11) to 223 cM (chromosome 1) (Table 2). This result is consistent with the lengths of these chromosomes as determined by the International Rice Genome Sequencing Project (2005): chromosomes 1 through 3 are larger

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**Figure 2.** The use of mPing-SCAR markers. (A) Schematic of a polymorphic locus. Grey triangles represent the inverted-repeat of mPing and arrows indicate the positions of locus specific primers. (B) An example of a mPing-SCAR marker. 100 bp-ladder (M) is followed by EG4 (G), Nipponbare (N) and the RILs. The gel image was visualized after 15 min running at 100 V.
than chromosomes 9 through 11. Moreover, by comparing the genetic distances of mPing-SCAR markers with their physical locations (according to the annotated rice genome) (see Supplementary Table S1), we find that the genetic distances strongly correlate with physical distances, thus providing another indication of the accuracy of mPing-SCAR markers (Table 2; see Supplementary Table S1).

As can be seen in Fig. 1, there are still chromosomal regions lacking markers (the short arm of chromosome 4), or with few markers (only two markers on the short arm of chromosome 9) (Fig. 1; see Supplementary Table S1). However, the upper half of chromosomes 4 and 9 including centromeres is known to be heterochromatic with low gene density (http://www.tigr.org/tdb/e2k1/osa1/). In addition, no SCAR markers were in centromeric, pericentromeric or telomeric regions, according to their physical positions in the annotated rice genome (see Supplementary Table S1). These facts strongly indicate that mPing markers in heterochromatic regions may not be a serious problem if gene discovery is the objective. However, a total of 926 insertion sites were already identified and some of them are located on such heterochromatic regions (Naito et al., unpublished data). Designing more primer sets to cover the whole genomic region is now underway.

3.5 QTL analysis of the heading date

To perform QTL analysis, days to heading of Nipponbare, EG4 and RIL populations were investigated in 2005 and 2007. The difference in days to heading was very small between these parental lines, with mean values for Nipponbare and EG4 of 98 and 103 days in 2005, and 102 and 104 days in 2007. However, the variation for days to heading of RILs showed transgressive segregation (range 93–111 days in 2005 and 99–110 days in 2007) and was continuous, suggesting a bimodal distribution (Fig. 3 and Supplementary Fig. S1). These results indicated that more than two loci with opposing effects were involved in the segregation of heading date in this cross. Both QTL analyses performed in 2005 and 2007 (with QTL chartographer) detected a single QTL peak (LOD score > 8.5) on the short arm of chromosome 6. Significant threshold LOD values (P < 0.05) in the F5 populations (2005) and in the F7 populations (2007) were 2.9 and 3.71, respectively. This peak was located within a 847 kb region between MK6_2 (2,034,336 nt) and RM3414 (2,881,883 nt) (Fig. 4). The additive effects of the Nipponbare allele at this locus were -1.9 (2005) and -2.2 (2007) days, respectively.

Although some genes concerning heading date such as Hd3a and RFT1 have been identified on short arm of chromosome 6, they are located in the interval between markers RM3414 and MK6_3 according to their sequences which is apparently excluded from the QTL peak detected in this study (Fig. 4).

Rather, this QTL locus fell into the same region of the recently identified locus Hd17.50 Hd17 was mapped using backcrossed inbred lines derived from Nipponbare (japonica) x Koshihikari (japonica), and the Koshihikari allele at this locus was indicated to increase the days to heading compared with Nipponbare allele by a few days. The Gimbozu allele identified in our study has almost the same effect on heading date versus Nipponbare, indicating that

### Table 2

| Molecular marker    | Number of markers on chromosome |
|---------------------|---------------------------------|
| mPing-SCAR markers  | 26 15 17 13 12 10 13 10 7 10 10 7 150 |
| SSR markers         | 5 5 3 5 2 1 5 7 2 2 4 4 46             |
| Total               | 31 20 20 18 14 11 19 17 9 13 14 10 196 |
| Chromosome length (cM) | 223 179 202 137 141 146 162 153 100 96 93 139 1,771 |
| Average intervals (cM) | 7.2 9.0 10.1 7.6 10.1 13.3 8.5 9.0 11.1 7.4 6.6 13.9 9.0 |

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![Figure 3. Histograms of days to heading in the F5 RIL populations. Horizontal bars indicate the values of parental Nipponbare and EG4.](image-url)
this locus is also *Hd17*. This fact also indicates that *mPing*-SCAR markers established here are comparable to large scale analysis using SNP/SSR markers.

In addition, it should be noted that classical genetic research had elucidated that the difference in flowering time between Gimbozu (including EG4) and Nipponbare is partly due to a flowering locus *E2*, with Gimbozu carrying an allele dominant (*E2*) to that of Nipponbare (*e2*).\(^5\) Koshihikari is also known to carry the dominant *E2* allele.\(^5\) Thus, Gimbozu and Koshihikari probably have the same allele at this locus. Matsubara et al.\(^5\) suggested that the *E2* locus corresponds to either *Hd16* (between 32 855–33 966 kb on chromosome 3) or *Hd17* (between 1964 and 2292 kb on chromosome 6). However, in the absence of information on chromosomal location, they were unable to determine which is the *E2* locus. In this study, however, we detected one QTL overlapping *Hd17* but none corresponding to *Hd16*. Taken together, we propose that *Hd17* is the previously described *E2* locus.\(^5\)\(^2\),\(^5\)\(^3\)

However, in 2005, we could not detect any other QTLs although the *F5* RILs showed transgressive segregation in heading date. This might be because *F5* populations should be segregating for traits, leading to disruption of data analysis. In fact, the analysis using *F7* RIL populations in 2007 detected some minor QTL peaks (LOD > 3.71) on chromosomes 1, 2, 3 and 12 (Supplementary Figure S2). Alternatively, the minor QTLs are in involved in response to environmental cues other than day length, such as temperature because the average temperature during the growing season was higher in 2005 than in 2007. In 2005, there were 68 days with average temperature of 25°C or higher while there were only 55 days in 2007. To further identify minor QTL loci, plants may need to be grown in growth chambers that can strictly control the growth conditions.

### 3.6 QTL analysis on culm length

We also conducted QTL analysis on culm length using 96 *F7* RILs derived from Nipponbare and EG4. The average plant heights of EG4 and Nipponbare were 84 and 70 cm, respectively. The continuous and transgressive segregation was also detected, indicating involvement of more than two loci (Fig. 5). Two putative QTLs were detected on chromosomes 3 and 10.
10 (Fig. 6A and B). The largest QTL (LOD score > 9.5) was near mPing-SCAR marker MK3_13 and the other (LOD score > 7.5) was near MK10_2. Significant threshold of LOD values (P < 0.05) was 3.14. The additive effects of these QTLs on plant height are −2.8 cm (chromosome 3, Nipponbare allele) and +2.4 cm (chromosome 10, Nipponbare allele). The transgressive segregation on the culm length can mainly be explained by these two loci, although minor QTL (LOD score > 3.14) was also detected (near MK9_4 on chromosome 9) (data not shown). Interestingly, no genes or QTL associated with plant height have been previously reported in these QTL regions. Thus, the use of mPing-SCAR markers may have led to the detection of novel loci or alleles within japonica cultivars.

3.7 In conclusions

In this study we report the construction of a linkage map, on which QTL analysis could successfully be performed, using RIL populations derived from a cross between Nipponbare (japonica) × Gimbozu EG4 (japonica) (Fig. 1; see Supplementary Table S1) and insertion site polymorphisms of the mPing transposon.37 Unlike other DNA markers used for temperate japonica crossings, mPing-SCAR markers were highly polymorphic and covered almost the whole genomic regions. Although Kwon et al.27 obtained 221 markers in total, not a few markers formed clusters and left many gaps of >30 cM as well. In contrast, we obtained 183 markers with no clusters and attained better coverage of genomic regions than ever. Especially, the Nipponbare and EG4 are closer than any other japonica/japonica cross combinations reported before. The ratio of polymorphisms in SSR markers between the two was only 7.0%, whereas 10% or higher frequency was obtained in most other combinations (See Table 1). Furthermore, mPing-SCAR markers allowed us to easily map the candidate loci in a very short time. For genotyping each marker, only 15 min electrophoresis in 1% agarose gel is required after PCR, which is apparently simpler and easier than any other molecular markers, such as SSR, SNP and RFLP.

It should be emphasized that all characterized japonica varieties (other than EG4) have, like Nipponbare, ~50 or fewer mPing copies.34,37 Thus, by crossing EG4 to other japonica varieties, linkage map construction and QTL analyses can easily be achieved. In this regard it is important to note the recent growing demand for rice with eating quality and the use of the japonica variety Koshihikari as a genetic resource in several molecular breeding projects.23,54 These studies should benefit from the addition of EG4 and mPing-SCAR markers to facilitate

the identification of loci or genes controlling eating quality. The mPing-SCAR markers may also be valuable in uncovering genetic ‘treasures’ that remain hidden in the results of classical genetic studies pursued in Japan until 1990s. During that time japonica × japonica cross combinations were widely and vigorously undertaken to identify loci that co-segregate with unique phenotypes or traits. At present, 926 mPing insertion sites in the EG4 genome are identified (Naito et al., unpublished data). When mPing-SCAR markers of this number become available, the average distance among these markers will be estimated to be 402 kb [=372 090 kb/926 markers (according to RAP-DB: http://rapdb.dna.affrc.go.jp/)]. Considering the gene density of 1 gene/11.8 kb [=31 439 genes/372 090 kb (by RAP-DB)] in the rice genome, the estimated gene numbers between markers will be 31.4.

Although the progress of next-generation sequencing techniques is remarkable today,55,56 only the sequence information of the whole genome is not enough to clone a gene responsible for a phenotype. However, delimiting candidate genes by ~30 using mPing-SCAR markers would make it much more efficient to detect the target gene in combination with these sequencing techniques and well-developed database information. Once the candidate region is determined, the few sequence polymorphisms among japonica cultivars also make it far easier to clone the target gene. We are confident that the novel marker system developed in this study will dramatically save time, money and manpower in molecular breeding of rice.

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Supplementary data: Supplementary data are available at www.dnaresearch.oxfordjournals.org.

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References

1. Yano, M. 2001, Genetic and molecular dissection of naturally occurring variation, Current Opinion in Plant Biology, 4, 130–135.
2. Ashikari, M. and Matsuoka, M. 2006, Identification, isolation and pyramiding of quantitative trait loci for rice breeding, Science, 11, 344–350.

3. Paterson, A. H., Lander, E. S., Hewitt, J. D., Peterson, S., Lincoln, S. E. and Tanksley, S. D. 1988, Resolution of quantitative traits into Mendelian factors by using a complete linkage map of restriction fragment length polymorphism, Nature, 335, 721–726.

4. Edwards, M. D., Helentjaris, T., Wright, S. and Stuber, C. W. 1992, Molecular-marker-facilitated investigations of quantitative trait loci in maize. IV. Analysis based on genome saturation with isozyme and restriction fragment length polymorphism markers, Theor. Appl. Genet., 83, 765–774.

5. Stuber, C. W., Lincoln, S. E., Wolff, D. W., Helentjaris, T. and Lander, E. S. 1992, Identification of genetics factors contributing to heterosis in a hybrid from two elite maize inbred lines using molecular markers, Genetics, 132, 823–839.

6. Martin, G. B., Brommonschenkel, S. H., Chunwongse, J., et al. 1993, Map-based cloning of a protein kinase gene conferring disease resistance in tomato, Science, 262, 1432–1436.

7. DeVicente, M. C. and Tanksley, S. D. 1993, QTL analysis of transgressive segregation in an interspecific tomato cross, Genetics, 134, 585–596.

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

9. Nishimura, A., Ashikari, M., Lin, S., et al. 2005, Isolation of a rice regeneration quantitative trait loci gene and its application to transformation systems, Proc. Natl Acad. Sci. USA, 102, 11940–1.

10. Harushima, Y., Yano, M., Shomura, A., et al. 1988, A high-density rice genetic linkage map with 2275 markers using a single F2 population, Genetics, 148, 479–494.

11. Kurata, N., Nagamura, Y., Yamamoto, K., et al. 1994, A 300 kilobase interval genetic map of rice including 883 expressed sequences, Nat. Genet., 8, 365–372.

12. McCouch, S. R., Teytelman, L., Xu, Y., et al. 2002, Development and mapping of 2240 new SSR markers for rice (Oryza sativa L.), DNA Res., 9, 199–207.

13. Yano, M., Katayose, Y., Ashikari, M., et al. 2000, Hd1, a major photoperiod sensitivity quantitative trait locus in rice, is closely related to the Arabidopsis flowering time gene CONSTANS, Plant Cell, 12, 2473–2483.

14. Takahashi, Y., Shomura, A., Sasaki, T. and Yano, M. 2001, Hd6, a rice quantitative trait locus involved in photoperiod sensitivity, encodes the α subunit of protein kinase CK2, Proc. Natl Acad. Sci. USA, 98, 7922–7927.

15. Kojima, S., Takahashi, Y., Kobayashi, Y., et al. 2002, Hd3a, a rice ortholog of the Arabidopsis FT gene, promotes transition to flowering downstream of Hd1 under short-day conditions, Plant Cell Physiol., 43, 1096–1105.

16. Doi, K., Izawa, T., Fuse, T., et al. 2004, Ehd1, a B-type response regulator in rice, confers short-day promotion of flowering and controls FT-like gene expression independently of Hd1, Genes Dev., 18, 926–936.

17. Ashikari, M., Jianzhong, W., Masahiro, Y., Sasaki, T. and Yoshimura, A. 1999, Rice geberelline-insensitive dwarf mutant gene Dwarf1 encodes the α-subunit of GTP-binding protein, Proc. Natl Acad. Sci. USA, 96, 10284–10289.

18. Kobayashi, A., Genliang, B., Shenghui, Y. and Tomita, K. 2007, Detection of quantitative trait loci for white-back and basal-white kernels under high temperature stress in japonica rice varieties, Breeding Science, 57, 107–116.

19. Nasu, S., Suzuki, J., Ohta, R., et al. 2002, Search for and analysis of single nucleotide polymorphisms (SNPs) in rice (Oryza sativa, Oryza rufipogon) and establishment of SNP markers, DNA Res., 9, 163–171.

20. Monna, L., Ohta, R., Masuda, H., Koike, A. and Minobe, Y. 2006, Genome-wide searching of single-nucleotide polymorphisms among eight distantly and closely related rice cultivars (Oryza sativa L.) and a wild accession (Oryza rufipogon Griff.), DNA Res., 13, 43–51.

21. Shirasawa, K., Maeda, H., Monna, L., Kishitake, S. and Nishio, T. 2007, The number of genes having different alleles between rice cultivars estimated by SNP analysis, Theor. Appl. Genet., 115, 1067–1074.

22. Takeuchi, Y., Hayasaka, H., Chiba, B., Tanaka, I. and Shimano, T. 2001, Mapping quantitative trait loci controlling cool-temperature tolerance at booting stage in temperate japonica rice, Breeding Science, 51, 191–197.

23. Wada, T., Uchimura, Y., Ogata, T., Tsubone, M. and Matsue, Y. 2006, Mapping of QTLs for physicochemical properties in japonica rice, Breeding Science, 56, 253–260.

24. Tabata, M., Hirabayashi, H., Takeuchi, Y., Ando, I., lida, Y. and Ohsawa, R. 2007, Mapping of quantitative trait loci for the occurrence of white-back kernels associated with high temperatures during the ripening period of rice (Oryza sativa L.), Breeding Science, 57, 47–52.

25. Takeuchi, Y., Hori, K., Suzuki, K., Nonoue, Y. and Takemoto-Kuno, Y. 2008, Major QTLs for eating quality of an elite Japanese rice cultivar, Koshihikari, on the short arm of chromosome 3, Breeding Science, 58, 437–445.

26. Kobayashi, A. and Tomita, K. 2008, QTL detection for stickiness of cooked rice using recombinant inbred lines derived from crosses between japonica rice cultivars, Breeding Science, 58, 419–426.

27. Kwon, S. J., Cho, Y. C., Kwon, S. W., et al. 2008, QTL mapping of agronomic traits using an RIL population derived from a cross between temperate japonica cultivars in rice (Oryza sativa L.), Breeding Science, 58, 271–279.

28. Casa, A. M., Brouwer, M., Nagel, A., et al. 2000, The MITE family Heartbreaker (Hbr): molecular markers in maize, Proc. Natl Acad. Sci. USA, 97, 10083–10089.

29. Jiang, N., Bao, Z., Temnykh, S., et al. 2002, Dasheng: a recently amplified nonautonomous long terminal repeat element that is a major component of pericentromeric regions in rice, Genetics, 161, 1293–1305.

30. Park, K. C., Lee, J. K., Kwon, S. J., et al. 2004, Distribution of MITE transposons in a rice genetic map, Korean J. Breed., 36, 95–102.

31. Kwon, S. J., Hong, S. W., Son, J. H., et al. 2006, CACTA and MITE transposon distributions on a genetic map of rice using F15 RILs derived from Milyang 23 and Gihobyeo hybrids, Mol. Cells., 21, 360–366.
32. Lee, J. K., Park, J. M., Kim, J. H., et al. 2006, Genetic mapping of the *Isaac*-CACTA transposon in maize, *Theor. Appl. Genet.*, 113, 16–22.

33. Huang, X., Lu, G., Zhao, Q., Liu, X. and Han, B. 2008, Genome-wide analysis of transposon insertion polymorphisms reveals intraspecific variation in cultivated rice, *Plant Physiol.*, 148, 25–40.

34. Jiang, N., Bao, Z., Zhang, X., et al. 2003, An active DNA transposon family in rice, *Nature*, 421, 163–167.

35. Kikuchi, K., Terauchi, K., Wada, M. and Hirano, H. 2003, The plant MITE *mPing* is mobilized in anther culture, *Nature*, 421, 167–170.

36. Nakazaki, T., Okumoto, Y., Horibata, A., et al. 2003, Mobilization of a transposon in the rice genome, *Nature*, 421, 170–172.

37. Naito, K., Cho, E., Yang, G., et al. 2006, Dramatic amplification of a rice transposable element during recent domestication, *Proc. Natl Acad. Sci. USA*, 103, 17620–17625.

38. Murray, M. G. and Thompson, W. F. 1980, Rapid isolation of high molecular weight plant DNA, *Nucleic Acid Res.*, 8, 4321–4325.

39. Lincoln, S. E., Daly, M. and Lander, E. S. 1992, Construction genetic maps with MAPMAKER/EXP 3.0, Whitehead Institute for Biomedical Research Technical Report: Cambridge, MA.

40. Zeng, Z. B. 1994, Precision mapping of quantitative trait loci, *Genetics*, 136, 1457–1468.

41. Basten, C. J., Weir, B. S. and Zeng, Z. B. 1998, QTL CARTOGRAPHER: a reference manual and tutorial for QTL mapping, Raleigh, NC: Department of Statistics, North Carolina State University.

42. Churchill, G. A. and Doerge, R. W. 1994, Empirical threshold values for quantitative trait mapping, *Genetics*, pp. 963–971. 138.

43. Engels, W. R., Johnson-Schlitz, D. M., Eggleston, W. B. and Sved, J. 1990, High-frequency *P* element loss in *Drosophila* is homolog dependent, *Cell*, 62, 515–525.

44. Lisch, D., Chomet, P. and Freeling, M. 1995, Genetic characterization of the *Mutator* system in maize: behavior and regulation of *Mu* transposons in a minimal line, *Genetics*, 139, 1777–1796.

45. Harushima, Y., Kurata, N., Yano, M., et al. 1992, Detection of segregation distortions in an *indica–japonica* rice cross using a high-resolution molecular map, *Theor. Appl. Genet.*, 92, 145–150.

46. International Rice Genome Sequencing Project. 2005, The map-based sequence of the rice genome, *Nature*, 436, 793–800.

47. Cheng, Z., Buell, C. R., Wing, R. A., Gu, M. and Jiang, J. 2001, Toward a cytological characterization of the rice genome, *Genome Res.*, 11, 2133–2141.

48. Feng, Q., Zhang, Y. J., Hao, P., et al. 2002, Sequence and analysis of rice chromosome 4, *Nature*, 420, 316–320.

49. Zhao, Q., Zhang, Y., Cheng, Z., et al. 2002, A fine map of the rice chromosome 4, *Genome Res.*, 12, 817–823.

50. Matsubara, K., Kono, I., Hori, K., et al. 2008, Novel QTLs for photoperiodic flowering revealed by using reciprocal backcross inbred lines from crosses between *japonica* rice cultivars, *Theor. Appl. Genet.*, 117, 935–945.

51. Okumoto, Y., Tanisaka, T. and Yamagata, H. 1992, Heading-time genes of the rice varieties grown in the Tohoku-Hokuriku region in Japan, *Jpn. J. Breed.*, 42, 121–135.

52. Yamagata, H. 1984, Heading-time genes of rice, *E1*, *E2* and *E3*, *Rice Genet. Newslett.*, 1, 100–101.

53. Yamagata, H., Okumoto, Y. and Tanisaka, T. 1986, Analysis of genes controlling heading time in Japanese rice, *Rice Genetics*, The Philippines: International Rice Research Institute, pp. 351–359.

54. Blakeney, A. B., Lewin, L. G. and Reinke, R. F. 2001, Quality rice for north Asia, Australia: Rural Industries Research and Development Corporation.

55. Blow, N. 2007, The personal side of genomics, *Nature*, 449, 627–630.

56. Hutchison, C. A. Ill 2007, DNA sequencing: bench to beside and beyond, *Nucleic Acids Res.*, 35, 6227–6237.